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Search Results -

Term	Documents
(3 NOT 4).USPT,PGPB,JPAB,EPAB,DWPI.	16
(L3 NOT L4).USPT,PGPB,JPAB,EPAB,DWPI.	16

Database: US Patents Full-Text Database
US Pre-Grant Publication Full-Text Database
JPO Abstracts Database
EPO Abstracts Database
Derwent World Patents Index
IBM Technical Disclosure Bulletins

Search: L5

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Search History

DATE: Monday, February 18, 2002 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L5	l3 not l4	16	L5
L4	L3 and capture and probe	9	L4
L3	L2 and hairpin and ribozyme	25	L3
L2	beta replicase	487	L2
L1	q(2$) replicase	0	L1

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 10 of 37 returned.

☐ 1. Document ID: US 6316612 B1

L6: Entry 1 of 37 File: USPT Nov 13, 2001

US-PAT-NO: 6316612
DOCUMENT-IDENTIFIER: US 6316612 B1

TITLE: Xylofuranosly-containing nucleoside phosphoramidites and polynucleotides

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 6307041 B1

L6: Entry 2 of 37 File: USPT Oct 23, 2001

US-PAT-NO: 6307041
DOCUMENT-IDENTIFIER: US 6307041 B1

TITLE: Circular, hairpin, circular/hairpin, lariat, and hairpin-lariat hammerhead ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 3. Document ID: US 6280936 B1

L6: Entry 3 of 37 File: USPT Aug 28, 2001

US-PAT-NO: 6280936
DOCUMENT-IDENTIFIER: US 6280936 B1

TITLE: Method for screening nucleic acid catalysts

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 4. Document ID: US 6251666 B1

L6: Entry 4 of 37 File: USPT Jun 26, 2001

US-PAT-NO: 6251666
DOCUMENT-IDENTIFIER: US 6251666 B1

TITLE: Nucleic acid catalysts comprising L-nucleotide analogs

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 5. Document ID: US 6248878 B1

L6: Entry 5 of 37 File: USPT Jun 19, 2001

US-PAT-NO: 6248878
DOCUMENT-IDENTIFIER: US 6248878 B1

TITLE: Nucleoside analogs

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☑ 6. Document ID: US 6221661 B1

L6: Entry 6 of 37 File: USPT Apr 24, 2001

US-PAT-NO: 6221661
DOCUMENT-IDENTIFIER: US 6221661 B1

TITLE: Hairpin ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 7. Document ID: US 6201113 B1

L6: Entry 7 of 37 File: USPT Mar 13, 2001

US-PAT-NO: 6201113
DOCUMENT-IDENTIFIER: US 6201113 B1

TITLE: Zymogenic nucleic acid molecules

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 8. Document ID: US 6183959 B1

L6: Entry 8 of 37 File: USPT Feb 6, 2001

US-PAT-NO: 6183959
DOCUMENT-IDENTIFIER: US 6183959 B1

TITLE: Method for target site selection and discovery

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 9. Document ID: US 6177075 B1

L6: Entry 9 of 37 File: USPT Jan 23, 2001

US-PAT-NO: 6177075
DOCUMENT-IDENTIFIER: US 6177075 B1

TITLE: Insect viruses and their uses in protecting plants

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

☐ 10. Document ID: US 6159951 A

L6: Entry 10 of 37 File: USPT Dec 12, 2000

US-PAT-NO: 6159951
DOCUMENT-IDENTIFIER: US 6159951 A

TITLE: 2'-O-amino-containing nucleoside analogs and polynucleotides

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

Generate Collection Print

Term	Documents
HAIRPIN.USPT.	5567
HAIRPINS.USPT.	796
RIBOZYME.USPT.	2966
RIBOZYMES.USPT.	2832
LIGAT$5	0
LIGAT.USPT.	3
LIGATABLE.USPT.	273
LIGATABLY.USPT.	4
LIGATATES.USPT.	1
LIGATATION.USPT.	2
LIGATCD.USPT.	1
(HAIRPIN SAME RIBOZYME SAME LIGAT$5).USPT.	37

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Search Results - Record(s) 11 through 20 of 37 returned.

☐ 11. Document ID: US 6140055 A

L6: Entry 11 of 37 File: USPT Oct 31, 2000

US-PAT-NO: 6140055
DOCUMENT-IDENTIFIER: US 6140055 A

TITLE: Zymogenic nucleic acid detection methods and related kits

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

☐ 12. Document ID: US 6132962 A

L6: Entry 12 of 37 File: USPT Oct 17, 2000

US-PAT-NO: 6132962
DOCUMENT-IDENTIFIER: US 6132962 A

TITLE: Retroviral vectors comprising an anti-hiv or other nucleic acid

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

☐ 13. Document ID: US 6103890 A

L6: Entry 13 of 37 File: USPT Aug 15, 2000

US-PAT-NO: 6103890
DOCUMENT-IDENTIFIER: US 6103890 A

TITLE: Enzymatic nucleic acids that cleave C-fos

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

☐ 14. Document ID: US 6096880 A

L6: Entry 14 of 37 File: USPT Aug 1, 2000

US-PAT-NO: 6096880
DOCUMENT-IDENTIFIER: US 6096880 A

TITLE: Circular DNA vectors for synthesis of RNA and DNA

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

☐ 15. Document ID: US 6077668 A

L6: Entry 15 of 37 File: USPT Jun 20, 2000

US-PAT-NO: 6077668
DOCUMENT-IDENTIFIER: US 6077668 A

TITLE: Highly sensitive multimeric nucleic acid probes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 16. Document ID: US 6057156 A

L6: Entry 16 of 37 File: USPT May 2, 2000

US-PAT-NO: 6057156
DOCUMENT-IDENTIFIER: US 6057156 A

TITLE: Enzymatic nucleic acid treatment of diseases or conditions related to levels of epidermal growth factor receptors

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 17. Document ID: US 6043077 A

L6: Entry 17 of 37 File: USPT Mar 28, 2000

US-PAT-NO: 6043077
DOCUMENT-IDENTIFIER: US 6043077 A

TITLE: Hepatitis C virus ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 18. Document ID: US 6022962 A

L6: Entry 18 of 37 File: USPT Feb 8, 2000

US-PAT-NO: 6022962
DOCUMENT-IDENTIFIER: US 6022962 A

TITLE: Hairpin ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 19. Document ID: US 5877162 A

L6: Entry 19 of 37 File: USPT Mar 2, 1999

US-PAT-NO: 5877162
DOCUMENT-IDENTIFIER: US 5877162 A

TITLE: Short external guide sequences

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 20. Document ID: US 5869339 A

L6: Entry 20 of 37 File: USPT Feb 9, 1999

US-PAT-NO: 5869339
DOCUMENT-IDENTIFIER: US 5869339 A

TITLE: HIV targeted hairpin ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

Generate Collection | Print

Term	Documents
HAIRPIN.USPT.	5567
HAIRPINS.USPT.	796
RIBOZYME.USPT.	2966
RIBOZYMES.USPT.	2832
LIGAT$5	0
LIGAT.USPT.	3
LIGATABLE.USPT.	273
LIGATABLY.USPT.	4
LIGATATES.USPT.	1
LIGATATION.USPT.	2
LIGATCD.USPT.	1
(HAIRPIN SAME RIBOZYME SAME LIGAT$5).USPT.	37

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Search Results - Record(s) 21 through 30 of 37 returned.

☐ 21. Document ID: US 5869248 A

L6: Entry 21 of 37 File: USPT Feb 9, 1999

US-PAT-NO: 5869248
DOCUMENT-IDENTIFIER: US 5869248 A

TITLE: Targeted cleavage of RNA using ribonuclease P targeting and cleavage sequences

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 22. Document ID: US 5866701 A

L6: Entry 22 of 37 File: USPT Feb 2, 1999

US-PAT-NO: 5866701
DOCUMENT-IDENTIFIER: US 5866701 A

TITLE: HIV targeted hairpin ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 23. Document ID: US 5858785 A

L6: Entry 23 of 37 File: USPT Jan 12, 1999

US-PAT-NO: 5858785
DOCUMENT-IDENTIFIER: US 5858785 A

TITLE: HIV targeted ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 24. Document ID: US 5856188 A

L6: Entry 24 of 37 File: USPT Jan 5, 1999

US-PAT-NO: 5856188
DOCUMENT-IDENTIFIER: US 5856188 A

TITLE: Hairpin ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 25. Document ID: US 5837855 A

L6: Entry 25 of 37 File: USPT Nov 17, 1998

US-PAT-NO: 5837855
DOCUMENT-IDENTIFIER: US 5837855 A

TITLE: Hairpin ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 26. Document ID: US 5834440 A

L6: Entry 26 of 37 File: USPT Nov 10, 1998

US-PAT-NO: 5834440
DOCUMENT-IDENTIFIER: US 5834440 A

TITLE: Ribozyme therapy for the inhibition of restenosis

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 27. Document ID: US 5811275 A

L6: Entry 27 of 37 File: USPT Sep 22, 1998

US-PAT-NO: 5811275
DOCUMENT-IDENTIFIER: US 5811275 A

TITLE: HIV-specific ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 28. Document ID: US 5763171 A

L6: Entry 28 of 37 File: USPT Jun 9, 1998

US-PAT-NO: 5763171
DOCUMENT-IDENTIFIER: US 5763171 A

TITLE: Nucleic acid structures with catalytic and autocatalytic replicating features and methods of use

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 29. Document ID: US 5670361 A

L6: Entry 29 of 37 File: USPT Sep 23, 1997

US-PAT-NO: 5670361
DOCUMENT-IDENTIFIER: US 5670361 A

TITLE: HIV-specific ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 30. Document ID: US 5667969 A

L6: Entry 30 of 37 File: USPT Sep 16, 1997

US-PAT-NO: 5667969
DOCUMENT-IDENTIFIER: US 5667969 A

TITLE: Alteration of sequence of a deleterious target molecule by ribozyme catalyzed trans-splicing

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

Generate Collection | Print

Term	Documents
HAIRPIN.USPT.	5567
HAIRPINS.USPT.	796
RIBOZYME.USPT.	2966
RIBOZYMES.USPT.	2832
LIGAT$5	0
LIGAT.USPT.	3
LIGATABLE.USPT.	273
LIGATABLY.USPT.	4
LIGATATES.USPT.	1
LIGATATION.USPT.	2
LIGATCD.USPT.	1
(HAIRPIN SAME RIBOZYME SAME LIGAT$5).USPT.	37

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Search Results - Record(s) 31 through 37 of 37 returned.

☐ 31. Document ID: US 5663064 A

L6: Entry 31 of 37 File: USPT Sep 2, 1997

US-PAT-NO: 5663064
DOCUMENT-IDENTIFIER: US 5663064 A

TITLE: Ribozymes with RNA protein binding site

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 32. Document ID: US 5646034 A

L6: Entry 32 of 37 File: USPT Jul 8, 1997

US-PAT-NO: 5646034
DOCUMENT-IDENTIFIER: US 5646034 A

TITLE: Increasing rAAV titer

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 33. Document ID: US 5633133 A

L6: Entry 33 of 37 File: USPT May 27, 1997

US-PAT-NO: 5633133
DOCUMENT-IDENTIFIER: US 5633133 A

TITLE: Ligation with hammerhead ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 34. Document ID: US 5631359 A

L6: Entry 34 of 37 File: USPT May 20, 1997

US-PAT-NO: 5631359
DOCUMENT-IDENTIFIER: US 5631359 A

TITLE: Hairpin ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 35. Document ID: US 5631115 A

L6: Entry 35 of 37 File: USPT May 20, 1997

US-PAT-NO: 5631115
DOCUMENT-IDENTIFIER: US 5631115 A

TITLE: Looped, hairpin ribozyme

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 36. Document ID: US 5527895 A

L6: Entry 36 of 37 File: USPT Jun 18, 1996

US-PAT-NO: 5527895
DOCUMENT-IDENTIFIER: US 5527895 A

TITLE: HIV targeted hairpin ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 37. Document ID: US 5472840 A

L6: Entry 37 of 37 File: USPT Dec 5, 1995

US-PAT-NO: 5472840
DOCUMENT-IDENTIFIER: US 5472840 A

TITLE: Nucleic acid structures with catalytic and autocatalytic replicating features and methods of use

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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Generate Collection | Print

Term	Documents
HAIRPIN.USPT.	5567
HAIRPINS.USPT.	796
RIBOZYME.USPT.	2966
RIBOZYMES.USPT.	2832
LIGAT$5	0
LIGAT.USPT.	3
LIGATABLE.USPT.	273
LIGATABLY.USPT.	4
LIGATATES.USPT.	1
LIGATATION.USPT.	2
LIGATCD.USPT.	1
(HAIRPIN SAME RIBOZYME SAME LIGAT$5).USPT.	37

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truncated HDV RNA fragments, which are different from the hammerhead or the **hairpin**/paperclip **ribozyme** model proposed for plant virusoid or virusoid RNAs, have been proposed. Whether these structures actually exist in vivo and whether ribozymes actually function in the HDV replication cycle have not been demonstrated. We have now developed an in vivo **ribozyme** self-cleavage assay capable of detecting self-cleavage of dimer or trimer HDV RNA in vivo. By site-directed mutagenesis and compensatory mutations to disrupt and restore potential base pairing in the **ribozyme** domain of the full-length HDV RNA according to the various structural models, a close correlation between the detected in vivo and the predicted in vitro **ribozyme** activities of various mutant RNAs was demonstrated. These results suggest that the proposed in vitro **ribozyme** structure likely exists and functions during the HDV replication cycle in vivo. Furthermore, the pseudoknot model most likely represents the structure responsible for the **ribozyme** activity in vivo. All of the mutants that had lost the **ribozyme** activity could not replicate, indicating that the **ribozyme** activities are indeed required for HDV RNA replication. However, some of the compensatory mutants which have restored both the cleavage and **ligation** activities could not replicate, suggesting that the **ribozyme** domains are also involved in other unidentified functions or in the formation of an alternative structure that is required for HDV RNA replication. This study thus established that the **ribozyme** has important biological functions in the HDV life cycle.

2/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08667638 96097008 PMID: 7495813
Kinetics and thermodynamics of intermolecular catalysis by **hairpin** ribozymes.
Hegg LA; Fedor MJ
Department of Biochemistry and Molecular Biology, University of Massachusetts Medical Center, Worcester 01655-0103, USA.
Biochemistry (UNITED STATES) Dec 5 1995, 34 (48) p15813-28, ISSN 0006-2960 Journal Code: A0G
Contract/Grant No.: GM 46422, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The **hairpin ribozyme**, derived from the negative strand of the satellite RNA of tobacco ringspot virus, belongs to the class of small catalytic RNAs that cleave RNA to generate 2',3'-cyclic phosphate and 5'hydroxyl termini and **ligate** these termini in the reverse reaction to form 3',5'-phosphodiesters. Rate and equilibrium constants for binding, dissociation, cleavage, and **ligation** steps in the kinetic mechanism were determined using a series of **hairpin ribozyme**/substrate pairs that differed in the sequence and length of the intermolecular base-paired helices. All **hairpin** variants cleaved with rate constants of approximately 0.3 min-1 at pH 7.5 in 10 nM MgCl2 at 25 degrees C, regardless of the length or sequence of the intermolecular helices. A rate constant of approximately 3 min-1 was determined for an intermolecular **ligation** reaction in which both cleavage products were supplied to the **ribozyme** in trans. Thus, the **hairpin** favored **ligation** over cleavage by 10-fold when the **ribozyme** was saturated with cleavage products. Binding rate constants for cleavage substrates and products were comparable to values reported for other catalytic RNAs but were somewhat slower than binding rates typical of small RNA helices. Substrate dissociation rate constants were much slower than cleavage rate constants for all substrates. Because virtually every substrate that was bound was cleaved before it could dissociate, KMS values were not the same as KdS values. Instead, KMS reflected the ratio of cleavage and substrate binding rate constants and had the same value of approximately 30 nM for all substrates. Calculations based on empirically determined free energy

parameters for simple RNA helices indicated that complexes between ribozymes and 5'-cleavage products were slightly less stable than simple helices with the same sequences. In contrast, affinities between ribozymes and cleavage substrates and between ribozymes and 3'-cleavage products were stronger than expected for simple duplexes by about -2.5 kcal/mol, evidence of stabilizing interactions in addition to those contributed by helical base pairs. This kinetic and thermodynamic study demonstrates that the kinetic mechanism of the **hairpin ribozyme** is distinct from the kinetic mechanisms of other well-characterized ribozymes and provides a foundation for further exploration of the **hairpin** structure and catalytic mechanism.

2/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08001578 93144312 PMID: 7678751
Ionic requirements for RNA binding, cleavage, and **ligation** by the **hairpin ribozyme**.
Chowrira BM; Berzal-Herranz A; Burke JM
Department of Microbiology and Molecular Genetics, Markey Center for Molecular Genetics, University of Vermont, Burlington 05405.
Biochemistry (UNITED STATES) Feb 2 1993, 32 (4) p1088-95, ISSN 0006-2960 Journal Code: A0G
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Metal ion requirements for RNA binding, cleavage, and **ligation** by the **hairpin ribozyme** have been analyzed. RNA cleavage is observed when Mg2+, Sr2+, or Ca2+ are added to a 40 mM Tris-HCl buffer, indicating that these divalent cations were capable of supporting the reaction. No reaction was observed when other ions (Mn2+, Co2+, Cd2+, Ni2+, Ba2+, Na+, K+, Li+, NH4+, Rb+, and Cs+) were tested. In the absence of added metal ions, spermidine can induce a very slow **ribozyme**-catalyzed cleavage reaction that is not quenched by chelating agents (EDTA and EGTA) that are capable of quenching the metal-dependent reaction. Addition of Mn2+ to a reaction containing 2 mM spermidine increases the rate of the catalytic step by at least 100-fold. Spermidine also reduces the magnesium requirement for the reaction and strongly stimulates activity at limiting Mg2+ concentrations. There are no special ionic requirements for formation of the initial **ribozyme**-substrate complex--analysis of complex formation using native gels and kinetic assays shows that the **ribozyme** can bind substrate in 40 mM Tris-HCl buffer. Complex formation is inhibited by both Mn2+ and Co2+. Ionic requirements for the **ribozyme**-catalyzed **ligation** reaction are very similar to those for the cleavage reaction. We propose a model for catalysis by the **hairpin ribozyme** that is consistent with these findings. Formation of an initial **ribozyme**-substrate complex occurs without the obligatory involvement of divalent cations. Ions (e.g., Mg2+) can then bind to form a catalytically proficient complex, which reacts and dissociates.(ABSTRACT TRUNCATED AT 250 WORDS)

2/3,AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07852677 93181189 PMID: 8441626
Cross-**ligation** and exchange reactions catalyzed by **hairpin** ribozymes.
Komatsu Y; Koizumi M; Sekiguchi A; Ohtsuka E
Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan.
Nucleic acids research (ENGLAND) Jan 25 1993, 21 (2) p185-90, ISSN 0305-1048 Journal Code: O8L
Languages: ENGLISH
Document type: Journal Article

Record type: Completed
The negative strand of the satellite RNA of tobacco ringspot virus (sTobRV(-)) contains a **hairpin** catalytic domain that shows self-cleavage and self-**ligation** activities in the presence of magnesium ions. We describe here that the minimal catalytic domain can catalyze a cross-**ligation** reaction between two kinds of substrates in trans. The cross-**ligated** product increased when the reaction temperature was decreased during the reaction from 37 degrees C to 4 degrees C. A two-stranded **hairpin ribozyme**, divided into two fragments between G45 and U46 in a **hairpin** loop, showed higher **ligation** activity than the nondivided **ribozyme** . The two stranded **ribozyme** also catalyzed an exchange reaction of the 3'-portion of the cleavage site.

2/3,AB/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07561407 92112029 PMID: 1730406
In vitro selection of active **hairpin** ribozymes by sequential RNA-catalyzed cleavage and **ligation** reactions.
Berzal-Herranz A; Joseph S; Burke JM
Department of Microbiology and Molecular Genetics, Markey Center for Molecular Genetics, University of Vermont, Burlington 05405.
Genes & development (UNITED STATES) Jan 1992, 6 (1) p129-34, ISSN 0890-9369 Journal Code: FN3
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
In vitro selection methods provide rapid and extremely powerful tools for elucidating interactions within and between macromolecules. Here, we describe the development of an in vitro selection procedure that permits the rapid isolation and evaluation of functional **hairpin** ribozymes from a complex pool of sequence variants containing an extremely low frequency of catalytically proficient molecules. We have used this method to analyze the sequence requirements of two regions of the **ribozyme** -substrate complex: a 7-nucleotide internal loop within the **ribozyme** that is essential for catalytic function and substrate sequences surrounding the cleavage-**ligation** site. Results indicate that only 3 of the 16,384 internal loop variants examined have high cleavage and **ligation** activity and that the **ribozyme** has a strong requirement for guanosine immediately 3' to the cleavage-**ligation** site.

2/3,AB/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07464039 92065960 PMID: 1956383
Novel guanosine requirement for catalysis by the **hairpin ribozyme**.
Chowrira BM; Berzal-Herranz A; Burke JM
Department of Microbiology and Molecular Genetics, Markey Center for Molecular Genetics, University of Vermont, Burlington 05405.
Nature (ENGLAND) Nov 28 1991, 354 (6351) p320-2, ISSN 0028-0836 Journal Code: NSC
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
THERE is much interest in the development of 'designer ribozymes' to target destruction of RNAs in vitro and in vivo. Engineering of ribozymes with novel specificities requires detailed knowledge of the **ribozyme** -substrate interaction, and a rigorous evaluation of sequence specificity. The **hairpin ribozyme** catalyses an efficient and reversible site-specific cleavage reaction. We have used mutagenesis and in vitro

selection strategies to show that RNA cleavage and **ligation** has an absolute requirement for guanosine immediately to the cleavage-**ligation** site. This G is not required for efficient substrate binding, rather, its 2-amino group is an essential component of the active site required for catalysis.

2/3,AB/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06976932 93173662 PMID: 1283911
Cross-**ligation** and exchange reaction of RNA catalyzed by **hairpin** ribozymes.
Komatsu Y; Koizumi M; Sekiguchi A; Ohtsuka E
Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan.
Nucleic acids symposium series (ENGLAND) 1992, (27) p43-4, ISSN 0261-3166 Journal Code: O8N
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The catalytic domain in the minus strand of the satellite RNA of tobacco ringspot virus (sTobRV(-)) assumes a **hairpin** -like secondary structure. This **ribozyme** catalyzes a cross-**ligation** reaction between substrate RNAs of different lengths. We constructed ribozymes to probe the activities of **ligation** and RNA fragment exchange.

2/3,AB/26 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12592190 BIOSIS NO.: 200000345692
Hairpin ribozymes.
AUTHOR: Chowrira Bharat(a); McSwiggen James A
AUTHOR ADDRESS: (a)Lafayette, CO**USA
JOURNAL: Official Gazette of the United States Patent and Trademark Office Patents 1231 (2):pNo pagination Feb. 8, 2000
MEDIUM: e-file
ISSN: 0098-1133
DOCUMENT TYPE: Patent
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: **Hairpin ribozyme** lacking a substrate moiety, comprising atleast six bases in helix 2 and able to base-pair with a separate substrate RNA, wherein the said **ribozyme** comprises one or more bases 3' of helix 3 able to base-pair with the said substrate RNA to form a helix 5 and wherein the said **ribozyme** can cleave and/or **ligate** said separate RNA(s) in trans.

2000

2/3,AB/27 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11047321 BIOSIS NO.: 199799668466
Methods in Molecular Biology, Vol. 74. **Ribozyme** protocols.
BOOK TITLE: Methods in Molecular Biology; **Ribozyme** protocols
AUTHOR: Turner Philip C
BOOK AUTHOR/EDITOR: Turner P C: Ed
AUTHOR ADDRESS: Univ. Liverpool, Liverpool**UK
JOURNAL: Methods in Molecular Biology 74pxiv+492p 1997
BOOK PUBLISHER: Humana Press Inc., Suite 808, 999 Riverview Drive, Totowa,

New [redacted]ersey 07512, USA
ISSN: 0097-0816 ISBN [redacted]0-89603-389-9
DOCUMENT TYPE: Book; Manual
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Each of the 49 individually authored chapters offers information on techniques for the use of ribozymes and is intended for molecular biologists, geneticists, virologists, and clinical researchers. The first part of the text describes the use of computers to select target sites and the use of libraries of **ribozyme** sequences to select those that will cleave or hybridize the target. The production of ribozymes by chemical methods and by using DNA templates and the modification and use of modified ribozymes are then discussed. Next the text describes the production of catalytic antisense RNAs, **hairpin** ribozymes, and RNase P ribozymes. The book then describes the theoretical and practical aspects of **ribozyme** mediated reactions including sections on characterizing **ribozyme** cleavage reactions and the determination of catalytic parameters for **hairpin** ribozymes. Methods of determining cleavage efficiency, including sections on the use of **ligation** -mediated PCR, transsplicing, and **ligation**, are then discussed. The text then details **ribozyme** structure and their delivery into cells. Finally, the book reviews the clinical use of ribozymes, specifically addressing heritable diseases and AIDS. The text contains figures, references, and a subject index.

1997

2/3,AB/28 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08125588 BIOSIS NO.: 000042106211
GUANOSINE-MEDIATED RNA CLEAVAGE AND **LIGATION** BY THE **HAIRPIN RIBOZYME**
AUTHOR: BURKE J; BERZAL-HERRANZ A; CHOWRIRA B; JOSEPH S
AUTHOR ADDRESS: UNIV. VT., BURLINGTON, VT. 05405.
JOURNAL: JOINT ANNUAL MEETING OF THE BIOPHYSICAL SOCIETY AND THE AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, HOUSTON, TEXAS, USA, FEBRUARY 9-13, 1992. BIOPHYS J 61 (2 PART 2). 1992. A411. 1992
CODEN: BIOJA
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH
1992

8/3,AB/30 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11739707 BIOSIS NO.: 199800520403
Molecular diagnosis of Chlamydia trachomatis infections by **probe** hybridization, PCR, LCR, TMA, and Q-**beta replicase**.
BOOK TITLE: Methods in Molecular Medicine; Sexually transmitted diseases: Methods and protocols
AUTHOR: Chernesky Max A; Mahony James B
BOOK AUTHOR/EDITOR: Peeling R W; Sparling P F: Eds
AUTHOR ADDRESS: Regional Virol. Chlamydia Lab., St. Joseph's Hosp., Hamilton, ON**Canada
JOURNAL: Methods in Molecular Medicine 20p33-46 1998
BOOK PUBLISHER: Humana Press Inc., Suite 808, 999 Riverview Drive, Totowa, New Jersey 07512, USA
ISBN: 0-89603-535-2
DOCUMENT TYPE: Book
RECORD TYPE: Citation
LANGUAGE: English
1998

8/3,AB/31 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11317540 BIOSIS NO.: 199800098872
Oligonucleotide **probe** technology as applied to the study of harmful algal blooms.
AUTHOR: Tyrrell John V; Bergquist Patricia R(a); Saul David J; MacKenzie Lincoln; Berquist Peter L
AUTHOR ADDRESS: (a)Cent. Marine Sci., Sch. Biol. Sci., Univ. Auckland, Private Bag 92 019, Auckland**New Zealand
JOURNAL: New Zealand Journal of Marine and Freshwater Research 31 (4):p 551-560 Dec., 1997
ISSN: 0028-8330
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Harmful algal bloom (HAB) research and monitoring has traditionally been based on ecological and microbiological measurements which are laborious, time-consuming, and reliant on experienced operators. Recent developments in oligonucleotide **probe** technology and immunofluorescence research have revealed several potential applications and techniques that may be transposable to laboratory and field-based monitoring and research. Field trials are currently underway for fluorescent in situ hybridization and sandwich hybridization assays. The former is particularly suited for laboratory-based research on harmful algal bloom (HAB) population dynamics and structure, whereas the sandwich hybridization assays based on a portable robotics workstation, offers the potential of quick and reliable laboratory and possibly field-based screening for HAB species. Initial development is underway for molecular beacons and the Qbeta replicase detection system, both offer the potential of simple and cost effective strategies for field-based monitoring by people with minimal knowledge of molecular biology.

1997

8/3,AB/32 (Item [redacted]rom file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11143348 BIOSIS NO.: 199799764493
Nucleic acid amplification methods for identifying cytogenetic abnormalities.
BOOK TITLE: Contemporary Biomedicine, 13; Human cytogenetic cancer markers
AUTHOR: O'Leary Timothy J
BOOK AUTHOR/EDITOR: Wolman S R; Sell S: Eds
AUTHOR ADDRESS: Dep. Cell Pathol., Armed Forces Inst. Pathol., Washington, DC**USA
p71-91 1997
BOOK PUBLISHER: Humana Press Inc., Suite 808, 999 Riverview Drive, Totowa, New Jersey 07512, USA
ISBN: 0-89603-357-0
DOCUMENT TYPE: Literature Review
RECORD TYPE: Citation
LANGUAGE: English
1997

8/3,AB/33 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10757964 BIOSIS NO.: 199799379109
Automation of molecular genetic methods - Part 2: DNA amplification techniques.
AUTHOR: Winn-Deen Emily S
AUTHOR ADDRESS: Applied Biosys. Div., Perkin Elmer Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404**USA
JOURNAL: Journal of Clinical Ligand Assay 19 (1):p21-26 1996
ISSN: 1081-1672
DOCUMENT TYPE: Literature Review
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: DNA amplification techniques can be divided into three groups: target amplification, **probe** amplification, and signal amplification. The target amplification techniques offer the highest level of detection sensitivity, with single molecule detection possible in finely tuned systems. This is followed closely by the **probe** amplification techniques, which can detect down to a few molecules of specific target sequence. Signal amplification has greatly improved through the use of branched probes and chemiluminescent detection and currently has a detection limit of 1000 molecules. Each of these amplification options is being commercialized in variety formats for use in the clinical laboratory. A description of the amplification techniques, approaches to their automation, and the clinical problems they address is reviewed in this article.

1996

8/3,AB/34 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09570702 BIOSIS NO.: 199598025620
Use of a Q-**beta-replicase** amplified **probe** assay and the isolator for the direct detection of Mycobacterium avium from blood.
AUTHOR: Nelson R; O'Brien W; Wlodyka M; Goldston L; Vera-Garcia M; Buxton D ; Stone B; Shah J; Dokidis A; King W; Weisburg W; Olive D M
AUTHOR ADDRESS: GENE-TRAK, Framingham, MA 01701**USA

JOURNAL: Abstracts of [redacted]e Interscience Conference on A[redacted]microbial Agents and Chemotherapy 34 [redacted]:p164 1994
CONFERENCE/MEETING: 34th Interscience Conference on Antimicrobial Agents and Chemotherapy Orlando, Florida, USA October 4-7, 1994
RECORD TYPE: Citation
LANGUAGE: English
1994

8/3,AB/35 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09274934 BIOSIS NO.: 199497283304
Loop-size variation to **probe** a bent structure of a **hairpin ribozyme**.
AUTHOR: Komatsu Yasuo; Koizumi Makoto; Nakamura Haruki; Ohtsuka Eiko(a)
AUTHOR ADDRESS: (a)Fac. Pharmaceutical Sciences, Hokkaido University, Sapporo 060**Japan
JOURNAL: Journal of the American Chemical Society 116 (9):p3692-3696 1994
ISSN: 0002-7863
DOCUMENT TYPE: Article
RECORD TYPE: Citation
LANGUAGE: English
1994

8/3,AB/36 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08631096 BIOSIS NO.: 199345049171
Rapid detection of human cytomegalovirus by an amplified assay using a ribozyme-activated "smart **probe**" and Q-**beta replicase**.
AUTHOR: Stefano K; McCarty J M; Rigby S; O'Brien W J; Stefano J E
AUTHOR ADDRESS: GENE-TRAK Systems, Framingham, MA 01701**USA
JOURNAL: Abstracts of the General Meeting of the American Society for Microbiology 93 (0):p486 1993
CONFERENCE/MEETING: 93rd General Meeting of the American Society for Microbiology Atlanta, Georgia, USA May 16-20, 1993
ISSN: 1060-2011
RECORD TYPE: Citation
LANGUAGE: English
1993

8/3,AB/37 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06451715 BIOSIS NO.: 000037023726
AMPLIFYING **PROBE** ASSAYS WITH Q-**BETA REPLICASE**
AUTHOR: KNIGHT P
JOURNAL: BIO-TECHNOLOGY (N Y) 7 (6). 1989. 609-610. 1989
FULL JOURNAL NAME: Bio-TECHNOLOGY (New York)
CODEN: BTCHD
RECORD TYPE: Citation
LANGUAGE: ENGLISH
1989

8/3,AB/38 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

02358801 BIOSIS NO [redacted]00065015826
CONFORMATIONAL ALTERATION OF PROTEIN SYNTHESIS ELONGATION FACTOR TU BY ELONGATION FACTOR TS AND BY KIRROMYCIN
AUTHOR: BLUMENTHAL T; DOUGLASS J; SMITH D
AUTHOR ADDRESS: DEP. MICROBIOL., INDIANA UNIV., BLOOMINGTON, INDIANA 47401, USA.
JOURNAL: PROC NATL ACAD SCI U S A 74 (8). 1977 3264-3267. 1977
FULL JOURNAL NAME: Proceedings of the National Academy of Sciences of the United States of America
CODEN: PNASA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Alterations of the structure of EF[elongation factor]-Tu were investigated by using the rate of EF-Tu cleavage by trypsin as a conformational **probe**. The presence of EF-Ts bound to EF-Tu resulted in a 10-fold increase in the cleavage rate. The antibiotic kirromycin, which inhibited protein synthesis by its interaction with EF-Tu, mimicked this effect of EF-Ts. Kirromycin and EF-Ts also facilitated the exchange of free GDP with GDP bound to EF-Tu. EF-Ts and kirromycin induced a similar conformational change in EF-Tu, thereby opening the guanine nucleotide binding site. The trypsin-cleaved EF-Tu still could bind GDP and EF-Ts and could function in Q.**beta**. **replicase**, but it no longer spontaneously renatured following denaturation in urea.

1977
? ds

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? b 155, 5

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     $0.00  Estimated cost File410
     $0.00  Estimated cost this search
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  File 155:MEDLINE(R)  1966-2002/Feb W2
  File   5:Biosis Previews(R)  1969-2002/Feb W2
         (c) 2002 BIOSIS

      Set  Items  Description
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? s beta (w) replicase and hairpin and ribozyme and capture and probe and
target

          953637  BETA
            3016  REPLICASE
             424  BETA(W)REPLICASE
            7843  HAIRPIN
            4617  RIBOZYME
           38883  CAPTURE
          158867  PROBE
          252316  TARGET
      S1       0  BETA (W) REPLICASE AND HAIRPIN AND RIBOZYME AND CAPTURE
                  AND PROBE AND TARGET
? s beta (w) replicase and hairpin and ribozyme and capture and probe

          953637  BETA
            3016  REPLICASE
             424  BETA(W)REPLICASE
            7843  HAIRPIN
            4617  RIBOZYME
           38883  CAPTURE
          158867  PROBE
      S2       0  BETA (W) REPLICASE AND HAIRPIN AND RIBOZYME AND CAPTURE
                  AND PROBE
? s beta (w) replicase and hairpin and ribozyme

          953637  BETA
            3016  REPLICASE
             424  BETA(W)REPLICASE
            7843  HAIRPIN
            4617  RIBOZYME
      S3       0  BETA (W) REPLICASE AND HAIRPIN AND RIBOZYME
? s beta (w) replicase

          953637  BETA
            3016  REPLICASE
      S4     424  BETA (W) REPLICASE
? s hairpin and ribozyme

            7843  HAIRPIN
            4617  RIBOZYME
      S5     497  HAIRPIN AND RIBOZYME
? s s4 and s5
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            424  S4
            497  S5
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            424  S4
            497  S5
         158867  PROBE
      S7     53  (S4 OR S5) AND PROBE
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...completed examining records
      S8     38  RD (unique items)
? t s8/3,ab/all


 8/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11235886   21226247   PMID: 11327881
   A base change in the catalytic core of the hairpin ribozyme
 perturbs function but not domain docking.
  Walter NG; Chan PA; Hampel KJ; Millar DP; Burke JM
  Department of Chemistry, The University of Michigan, Ann Arbor, Michigan
48109-1055, USA. nwalter@umich.edu
  Biochemistry (United States)  Feb 27 2001,  40  (8)  p2580-7, ISSN
0006-2960  Journal Code: A0G
  Languages: ENGLISH
  Document type: Journal Article
  Record type: In Process
  The hairpin ribozyme is a small endonucleolytic RNA motif
with potential for targeted RNA inactivation. It optimally cleaves
substrates containing the sequence 5'-GU-3' immediately 5' of G.
Previously, we have shown that tertiary structure docking of its two
domains is an essential step in the reaction pathway of the hairpin
ribozyme . Here we show, combining biochemical and fluorescence
structure and function probing techniques, that any mutation of the
substrate base U leads to a docked RNA fold, yet decreases cleavage
activity. The docked mutant complex shares with the wild-type complex a
common interdomain distance as measured by time-resolved fluorescence
resonance energy transfer (FRET) as well as the same solvent-inaccessible
core as detected by hydroxyl-radical protection; hence, the mutant complex
appears nativelike. FRET experiments also indicate that mutant docking is
kinetically more complex, yet with an equilibrium shifted toward the docked
conformation. Using 2-aminopurine as a site-specific fluorescent
probe in place of the wild-type U, a local structural rearrangement
in the substrate is observed. This substrate straining accompanies global
domain docking and involves unstacking of the base and restriction of its
conformational dynamics, as detected by time-resolved 2-aminopurine
fluorescence spectroscopy. These data appear to invoke a mechanism of
functional interference by a single base mutation, in which the
ribozyme -substrate complex becomes trapped in a nativelike fold
preceding the chemical transition state.


 8/3,AB/2     (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10765356   20452228   PMID: 10999599
  Solution structure and metal-ion binding of the P4 element from bacterial
RNase P RNA.
  Schmitz M; Tinoco I
  Department of Chemistry, University of California, Berkeley 94720-1460,
USA.
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RNA (UNITED STATES) [redacted]Sep 2000, 6 (9) p1212-25, [redacted]N 1355-8382
Journal Code: CHB
Contract/Grant No.: GM-10840, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

We determined the solution structure of two 27-nt RNA hairpins and their complexes with cobalt(III)-hexammine (Co(NH3)3+(6)) by NMR spectroscopy. The RNA hairpins used in this study are the P4 region from Escherichia coli RNase P RNA and a C-to-U mutant that confers altered divalent metal-ion specificity (Ca2+ replaces Mg2+) for catalytic activity of this **ribozyme**. Co(NH3)3+(6) is a useful spectroscopic **probe** for Mg(H2O)2+(6)-binding sites because both complexes have octahedral symmetry and have similar radii. The thermodynamics of binding to both RNA hairpins was studied using chemical shift changes upon titration with Mg2+, Ca2+, and Co(NH3)3+(6). We found that the equilibrium binding constants for each of the metal ions was essentially unchanged when the P4 model RNA **hairpin** was mutated, although the NMR structures show that the RNA hairpins adopt different conformations. In the C-to-U mutant a C.G base pair is replaced by U.G, and the conserved bulged uridine in the P4 wild-type stem shifts in the 3' direction by 1 nt. Intermolecular NOE cross-peaks between Co(NH3)3+(6) and RNA protons were used to locate the site of Co(NH3)3+(6) binding to both RNA hairpins. The metal ion binds in the major groove near a bulge loop, but is shifted 5' by more than 1 bp in the mutant. The change of the metal-ion binding site provides a possible explanation for changes in catalytic activity of the mutant RNase P in the presence of Ca2+.

8/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09723436 98183584 PMID: 9522983
Update on techniques in the diagnosis of Chlamydia trachomatis infections.
Ngan CC
Department of Pathology, Singapore General Hospital, Singapore.
Annals of the Academy of Medicine, Singapore (SINGAPORE) Nov 1997, 26 (6) p801-7, ISSN 0304-4602 Journal Code: 53F
Languages: ENGLISH
Document type: Journal Article; Review; Review, Tutorial
Record type: Completed

There are two major advances in the laboratory diagnosis of Chlamydia trachomatis, one lies in the use of nucleic acid amplification techniques and the second in the evaluation of urine as an alternative to invasive sampling of urethral and cervical specimens. There is however, the problem of inhibitors in urine that needs to be addressed, in order for this method to achieve 100% sensitivity. The Q-beta (Q **beta**) **replicase** test, Gen-**Probe** transcription-mediated amplification (TMA) test and the nucleic acid sequence-based amplification (NASBA) test are some of the newer nucleic acid amplification methods being evaluated for the detection of C. trachomatis. These are RNA-based amplification techniques that can potentially achieve very high levels of sensitivity because of the presence of multiple RNA copies in microorganisms and may also be useful for detecting active infection. Q-beta has been found to be less subjected to inhibitory substances in urine than polymerase chain reaction (PCR). Cell culture remains the gold standard for the legal diagnosis of C. trachomatis infections and is the method of choice for the detection of infection at uncommon sites. It forms part of the expanded gold standard for the evaluation of nonculture methods that do not involve nucleic acid amplification, and is also a confirmatory test for such techniques. Notwithstanding, clinicians must remember the basic tenet of laboratory tests, that is, good specimen collection and handling, for any laboratory test to yield accurate information to guide their management of patients.

8/3,AB/4 (Item [redacted]rom file: 155)
DIALOG(R)File 155:MEDLINE(R)

09721691 98164112 PMID: 9500810
Rapid and sensitive detection of Chlamydia trachomatis using a ligatable binary RNA **probe** and Q **beta replicase**.
Stefano JE; Genovese L; An Q; Lu L; McCarty J; Du Y; Stefano K; Burg JL; King W; Lane DJ
GENE-TRAK, Inc., Framingham, MA 01701, USA.
Molecular and cellular probes (ENGLAND) Dec 1997, 11 (6) p407-26, ISSN 0890-8508 Journal Code: NG9
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
A simple assay format was developed for the direct detection of C. trachomatis rRNA utilizing ligation of recombinant MDV-1 **probe** RNA fragments hybridized to 23S rRNA after capture and release from a solid support. Assay background (equivalent to 10(4) targets) was suppressed by blocking sequences in the 5' MDV reporter **probe** fragment complementary to the 3' fragment by prehybridization of a DNA oligonucleotide. A pair of reporter fragments bearing a deletion within the region, obtained by a hydrid-selection-amplification protocol, yielded a low level of assay background which was reduced to < 2% with a blocker directed against the remaining pairing sequence. This **probe** set showed a sensitivity of 10(3) molecules of 23S rRNA (> 95% responding) and could detect a single elementary body (EB) of Chlamydia trachomatis or 1-10 EB added to a clinical matrix of pooled negative human cervical swab samples. The time of first appearance of amplification products by real-time fluorescence detection showed a linear response to log increases in the target level over a 10(5)-fold range, permitting the determination of target level within an order of magnitude. The assay showed approximately 10(9)-fold discrimination over Chlamydia pneumonae (TWAR) rRNA. High levels of cultured C. albicans, E. coli, S. aureus, or N. gonorrhoeae had no detectable effect on assay background or the ability to detect a single elementary body.

8/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09617937 98022905 PMID: 9356249
Probing RNA-protein interactions using pyrene-labeled oligodeoxynucleotides: Qbeta replicase efficiently binds small RNAs by recognizing pyrimidine residues.
Preuss R; Dapprich J; Walter NG
Department of Biochemical Kinetics, Max-Planck-Institute for Biophysical Chemistry, Am Fassberg, Gottingen, D-37077, Germany.
Journal of molecular biology (ENGLAND) Oct 31 1997, 273 (3) p600-13, ISSN 0022-2836 Journal Code: J6V
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Binding of small RNAs by the RNA-dependent RNA polymerase of coliphage Qbeta was studied utilizing a fluorometric assay. A DNA oligonucleotide **probe** of sequence 5'-d(TTTTTCC) was 5'-end-labeled with pyrene. In this construct, the proximal thymine residues efficiently quench the fluorophore emission in solution. Upon stoichiometric binding of one **probe** per polymerase molecule, the pyrene steady-state fluorescence increases by two orders of magnitude, the fluorescence anisotropy increases, and a long fluorescence lifetime component of 140 ns appears. With addition of replicable RNA, steady-state fluorescence decreases in a concentration dependent manner and the long lifetime component is lost. This observation most likely reflects displacement of the pyrene-labeled **probe** from the proposed nucleic acid binding site II of Qbeta

replicase. The effe[redacted] was utilized to access bi[redacted]ng affinities of different RNAs to this site in a reverse titration assay format. In 10 mM sodium phosphate (pH 7.0), 100 mM NaCl, at 16 degrees C, equilibrium dissociation constants for different template midi- and minivariant RNAs were calculated to be in the nanomolar range. In general, the minus and plus strands, concomitantly synthesized by Qbeta replicase during replication, exhibited discriminative affinities, while their hybrid bound less efficiently than either of the single strands. Different non-replicable tRNAs also bound to the polymerase with comparable dissociation constants. By titration with DNA homo-oligonucleotides it was shown that the probed site on Qbeta replicase does not require a 2' hydroxyl group for binding nucleic acids, but recognizes pyrimidine residues. Its interaction with thymine is lost in an A.T base-pair, while that with cytosine is retained after Watson-Crick base-pairing. These findings can explain the affinities of RNA-Qbeta replicase interactions reported here and in earlier investigations. The sensitivity of the described fluorometric assay allows detection of RNA amplification by Qbeta replicase in real-time. Copyright 1997 Academic Press Limited.

8/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09541864 97376507 PMID: 9232617
Amplifiable hybridization probes containing a molecular switch.
Blok HJ; Kramer FR
Department of Molecular Genetics, Public Health Research Institute, New York, NY 10016, USA.
Molecular and cellular probes (ENGLAND) Jun 1997, 11 (3) p187-94, ISSN 0890-8508 Journal Code: NG9
Contract/Grant No.: AI-37015, AI, NIAID; HL-43521, HL, NHLBI
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
In order to reduce background signals in Q **beta replicase** -mediated bioassays, a target-dependent **probe** amplification strategy has been proposed that utilizes recombinant RNA hybridization probes that contain an inserted molecular switch. A molecular switch is an internal region of the **probe** that undergoes a conformational change when the **probe** hybridizes to its target. We investigated whether non-hybridized probes (which cause background signals) could be selectively destroyed by incubating the **probe**-target hybrids with ribonuclease III, which should cleave the non-hybridized probes and leave the hybridized probes intact. Two problems with this assay design were observed. First, ribonuclease III cleaved **probe**-target hybrids non-specifically when the target was an RNA, thereby destroying all of the bound probes. And second, the expected conformational change in the molecular switch did not occur when the probes were bound to their targets, apparently because the hairpin stem formed by the molecular switch was too long. Although these results demonstrated that the original assay design could not work, they provided insights that have led to better designs for target-dependent amplification assays. In these assays, the probes will be DNA molecules containing short-stemmed molecular switches. Non-hybridized probes will be selectively destroyed by incubation with a restriction endonuclease.

8/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09529963 97305999 PMID: 9163467
Performance of an automated Q-**beta replicase** amplification assay for Mycobacterium tuberculosis in a clinical trial.
Smith JH; Radcliffe G; Rigby S; Mahan D; Lane DJ; Klinger JD
Vysis Inc., Downers Grove, Illinois 60515, USA.
Journal of clinical microbiology (UNITED STATES) Jun 1997, 35 (6)

p1484-91, ISSN 009[redacted]137 Journal Code: HSH
Languages: ENGLISH
Document type: Clinical Trial; Journal Article; Multicenter Study
Record type: Completed
We present data from a clinical trial study in which an automated version (Galileo) of a previously described Q-**Beta replicase**-amplified **probe** assay (J. S. Shah et al., J. Clin. Microbiol. 33:1435-1441, 1995) was used for the direct detection of Mycobacterium tuberculosis complex in sputum. The assay was designed to target specific regions of 23S rRNA found in M. tuberculosis, Mycobacterium bovis, Mycobacterium africanum, and Mycobacterium microti and had a sensitivity ranging from approximately <10 to 300 CFU. The assay was tested for cross-hybridization by using large numbers (e.g., 10(5)to 10(10) CFU/assay) of 133 other organisms commonly found in respiratory tract samples, including non-M. tuberculosis Mycobacterium spp., other bacteria, fungi, and viruses. All of these competitors tested negative by the assay. Automated assay results for 780 respiratory tract samples (sputum or bronchoalveolar lavage specimens) collected and tested at three trial sites in the United States) were compared with the results of culture and acid-fast microscopy. Aliquots of conventionally digested and decontaminated sputum pellets were heated at 100 degrees C and mechanically disrupted prior to hybridization and background reduction, amplification, and detection in a closed disposable test pack. Pertinent elements of individual patient histories relating to tuberculosis exposure, previous active disease, antituberculosis therapy status, etc., were considered in the resolution of discrepant results for 48 (assay false-positive) samples. Seventy-one of 90 (78.9%) culture-positive samples were positive when tested in the Galileo assay, while 7% of culture-negative samples were assay positive, corresponding to a sensitivity of 79% and a specificity of 93%. Following resolution of discrepant results by chart review, the sensitivity and specificity for the Q-**Beta replicase** amplification assay with the Galileo analyzer were 84 and 97%, respectively. A total of 69.2% of smear-negative (culture positive) samples were detected by the assay. Ten test packs at a time were automatically processed by the Galileo analyzer without operator intervention following loading of samples. The first result was reported in approximately 3 h, and the last result was available in 6.5 h. To our knowledge, this is the first report of a clinical study with a fully automated amplification **probe** hybridization assay for the detection of pathogens directly from a clinical specimen.

8/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09344954 97305998 PMID: 9163466
Detection of Mycobacterium tuberculosis directly from sputum by using a prototype automated Q-**beta replicase** assay.
Smith JH; Buxton D; Cahill P; Fiandaca M; Goldston L; Marselle L; Rigby S ; Olive DM; Hendricks A; Shimei T; Klinger JD; Lane DJ; Mahan DE
Vysis Inc., Downers Grove, Illinois 60515, USA.
Journal of clinical microbiology (UNITED STATES) Jun 1997, 35 (6) p1477-83, ISSN 0095-1137 Journal Code: HSH
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
We have adapted an assay for the direct detection of Mycobacterium tuberculosis using a prototype automated instrument platform in which probes are amplified with Q-**beta replicase**. The assay was based on amplification of specific detector **probe** following four cycles of background reduction (reversible target capture) in a closed disposable pack. The assay signal was the time required for fluorescence to exceed background levels (response time [RT]). RT was inversely related to the number of M. tuberculosis rRNA target molecules in the sample. Equivalent signals and noises were observed in assays containing either sputum or buffer. All mock samples containing > or = 10 CFU of M. tuberculosis

responded in the assay (average RT, 13.91 min), while most (83%) samples containing as many as 10(7) CFU of Mycobacterium avium gave no response during a 25-min amplification reaction. The samples containing M. avium which did respond had an average RT of 17.04 min. Seventy-five percent (167 of 223) of samples containing no target gave no responses; the remaining 25% had an average RT of 15.53 min. Eighty-three frozen sputum samples were tested to develop a candidate cutoff RT for the assay prior to more extensive clinical testing. After resolution of discrepant results and with a 14-min RT cutoff, 30 of 38 M. tuberculosis-positive samples were positive by the assay; 1 of 45 negative samples responded within 14 min. Assay sensitivity, specificity, and positive and negatives predictive values in this pilot study were 79, 98, 97, and 85%, respectively.

8/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09119291 97067478 PMID: 8910891
Detection of rRNA from four respiratory pathogens using an automated Q **beta replicase** assay.
Stone BB; Cohen SP; Breton GL; Nietupski RM; Pelletier DA; Fiandaca MJ; Moe JG; Smith JH; Shah JS; Weisburg WG
GENE-TRAK, Framingham, MA 01701, USA.
Molecular and cellular probes (ENGLAND) Oct 1996, 10 (5) p359-70, ISSN 0890-8508 Journal Code: NG9
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Ribosomal RNA targets from Mycobacterium avium complex (23S), Mycoplasma pneumoniae (16S), Pneumocystis carinii (18S) and Legionella pneumophila (16S) were detected in four separate assays on a model automated Q-beta amplification instrument. Sandwich hybridization, reversible target capture, detector **probe** amplification and fluorescent signal detection occurred in closed, disposable packs at 38 degrees C. Packs were injected with 0.5 ml samples in 3.06 M guanidine thiocyanate. Ten samples per run were read after 7 h, requiring only 4 min loading time. Synthetic RNA transcripts and purified, natural RNAs from up to four different strains per assay were diluted to 10(6) or fewer molecules per sample (approximately 100 cells for prokaryotes, 10 cells for Pneumocystis). All analytes were detected at 10(6) targets. The limits of detection were found at 10(5) to 10(4). Discrimination against competitor RNA was tested using up to 10(9) molecules (1000 X excess) of appropriate test strains. Samples containing either zero targets or 10(7) competitors produced negative results in 95 to 100% of the samples, depending on the assay. Closely related Legionella and Mycoplasma species cross-reacted at high challenge levels of 10(9) molecules as a result of sequence similarities in the target regions. These results demonstrate the utility and versatility of an automated, high sensitivity, closed system for amplified analysis of direct-from-sample testing of respiratory pathogens.

8/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09069727 97018558 PMID: 8865174
Single molecule detection of RNA reporter probes by amplification with Q **beta replicase**.
Burg JL; Juffras AM; Wu Y; Blomquist CL; Du Y
GENE-TRAK Corp, Framingham, MA 01701, USA.
Molecular and cellular probes (ENGLAND) Aug 1996, 10 (4) p257-71, ISSN 0890-8508 Journal Code: NG9
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The addition of target-specific **probe** sequences within MDV RNA, an

otherwise efficient [redacted]mplate for Q **beta replicase**, [redacted]erally resulted in RNA molecules with inferior replication properties, including reduced replication rates and poor sensitivities. We have discovered that the replication characteristics of MDV RNA molecules with internally placed **probe** sequences are dramatically affected by short RNA sequences (spacer elements) at the 5' and 3' ends of the **probe** sequence. For a given **probe** sequence, the sequences of the flanking spacer elements effected replication sensitivity by six orders of magnitude and replication rate by three fold. By taking advantage of spacer elements, internal MDV probes were developed that permitted the reproducible, real time, fluorescence detection of a single RNA molecule in less than 25 min through amplification with Q **beta replicase**. RNA structural analysis of such probes suggested that the spacer elements functioned by allowing the RNA to fold in a way which substantially maintained the tertiary structure of the MDV domain. MDV reporter probes with suitable replication properties were obtained from libraries of RNA molecules in which the **probe** sequence was flanked by many different spacer elements (generated by random nucleotide synthesis). We demonstrated that this is a general method for developing RNA reporter molecules which are rapidly and reproducibly amplified by Q **beta replicase**, even from a single molecule.

8/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08673368 96099899 PMID: 7503417
Real-time fluorescence detection of RNA amplified by Q **beta replicase**.
Burg JL; Cahill PB; Kutter M; Stefano JE; Mahan DE
GENE-TRAK Corporation, Framingham, Massachusetts 01701, USA.
Analytical biochemistry (UNITED STATES) Sep 20 1995, 230 (2) p263-72
, ISSN 0003-2697 Journal Code: 4NK
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Amplification of RNA probes by Q **beta replicase** can be used to detect a wide range of analytes with a potential sensitivity of a single molecule. A system has been developed in which Q beta amplification of midivariant-(MDV)-based RNA is measured in real time by fluorescence. This was accomplished by including a fluorescent intercalating dye, propidium iodide, in the reactions and monitoring the fluorescence change using a custom fluorometer. The time at which fluorescence is detectable above background is referred to as the "response time" and is calculated using curve-fitting algorithms. A response time is inversely and linearly proportional to the logarithm of the number of template RNA molecules which initiated the reaction. Therefore, this system permits an unknown amount of input RNA **probe** to be quantified through 11 orders of magnitude when compared to a standard curve. Under the described conditions with MDV RNA, the response time occurs when about 3 x 10(11) RNA molecules are synthesized and occurs within the exponential phase of the reaction, before the number of active enzyme molecules are saturated with RNA templates. This system has been used to determine the replication properties of MDV RNA reporter molecules bearing specific **probe** sequences and to develop hybridization assays for the clinical diagnostic field.

8/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08592422 95378426 PMID: 7650163
Q-**beta replicase** -amplified assay for detection of Mycobacterium tuberculosis directly from clinical specimens.
Shah JS; Liu J; Buxton D; Hendricks A; Robinson L; Radcliffe G; King W; Lane D; Olive DM; Klinger JD
GENE-TRAK, Framingham, Massachusetts 01701, USA.

Journal of clinical microbiology (UNITED STATES) Jun 1995, 33 (6) p1435-41, ISSN 0095-1137 Journal Code: HSH
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

We report the results of a study conducted to evaluate the performance of manual Q-**Beta replicase** -amplified Mycobacterium tuberculosis complex assay compared with that of culture for detecting M. tuberculosis directly from digested sputum pellets. A total of 261 specimens submitted to three tuberculosis testing laboratories were analyzed. Culture and acid-fast bacillus smear results were provided by the tuberculosis testing laboratories. Of these 261 specimens, 34 (13% prevalence rate) were positive for M. tuberculosis by culture. The samples were digested and decontaminated by the testing laboratories by using their standard digestion and decontamination procedures. An aliquot of the digested and decontaminated pellet was sent to GENE-TRAK. The digested and decontaminated pellet was neutralized by washing it with 0.067 M phosphate buffer (pH 6.8), and the bacteria present in the washed pellet were heat inactivated at 100 degrees C for 15 min. The samples were combined with sample processing buffer containing GuSCN and were treated for 6 min in the GENE-TRAK Sample Processing Instrument to release the nucleic acids. The release rRNA was analyzed in a manual Q-**Beta replicase** assay format which incorporates elements of sandwich hybridization, reversible target capture, and Q-**beta replicase** signal amplification technologies. In comparison with culture, the overall assay sensitivity and specificity were 97.1 and 96.5%, respectively. The positive predictive value was 80.5%, and the negative predictive value was 99.5%. After analysis of discrepant results, the assay sensitivity and specificity were 97.3 and 97.8, respectively, and the prevalence rate was 14%. The positive predictive value and the negative predictive value were 87.8 and 99.5%, respectively.(ABSTRACT TRUNCATED AT 250 WORDS)

8/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08588330 95310453 PMID: 7540627

Comparison of amplified Q **beta replicase** and PCR assays for detection of Mycobacterium tuberculosis.

An Q; Buxton D; Hendricks A; Robinson L; Shah J; Lu L; Vera-Garcia M; King W; Olive DM
GENE-TRAK, Framingham, Massachusetts 01701, USA.

Journal of clinical microbiology (UNITED STATES) Apr 1995, 33 (4) p860-7, ISSN 0095-1137 Journal Code: HSH
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

Because of the long time required to isolate Mycobacterium tuberculosis in culture, there is an acute need for simple rapid methods for direct detection of M. tuberculosis from human sputum specimens. We have developed and characterized quantitative manual Q **beta replicase** and PCR assays for M. tuberculosis. The Q **beta replicase** assay was based on reversible target capture of M. tuberculosis 23S rRNA followed by amplification of a replicatable detector **probe** with Q **beta replicase** . For PCR assays, primers generating a 370-bp amplification product from the IS6110 insertion element were used in combination with a control plasmid containing an internal deletion in the IS6110 amplicon. Serial dilutions of M. tuberculosis were spiked into sputum and subjected to digestion and decontamination with N-acetyl-L-cysteine and NaOH. Assay conditions were optimized for hybridization and sample processing chemistries in order to maximize sample utilization. Following assay optimization, the sensitivities of the Q **beta replicase** and PCR assays of spiked sputum samples were 0.5 and 5.0 CFU per assay reaction, respectively. The effects of sputum matrix on each assay were examined by testing 20 patient sputum samples which had been cultured for M.

tuberculosis. The [redacted]ture-positive samples inclu smear-positive and smear-negative samples. The results of the Q **beta replicase** assay were not inhibited by sputum and were in 100% agreement with those of culture, including detection of 10 culture-positive specimens. However, using an internal control plasmid coamplified with each PCR as an indicator, we detected PCR inhibition in 9 of 20 samples tested.(ABSTRACT TRUNCATED AT 250 WORDS)

8/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08484775 95213382 PMID: 7699067
Comparison of characteristics of Q **beta replicase**-amplified assay with competitive PCR assay for Chlamydia trachomatis.
An Q; Liu J; O'Brien W; Radcliffe G; Buxton D; Popoff S; King W; Vera-Garcia M; Lu L; Shah J; et al
Gene-Trak, Framingham, Massachusetts 01701.
Journal of clinical microbiology (UNITED STATES) Jan 1995, 33 (1) p58-63, ISSN 0095-1137 Journal Code: HSH
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
In order to study infections due to Chlamydia trachomatis, we have compared semiquantitative PCR and Q **beta replicase**-amplified assays for detection of this organism. The PCR assay was directed against the C. trachomatis 16S rRNA gene. Quantitation was accomplished by adding known amounts of a plasmid containing a truncated segment of the 16S rRNA gene target to chlamydia-containing samples and then amplifying with a common primer set. The Q **beta replicase** assay consisted of reversible target capture of C. trachomatis 16S rRNA, which was followed by amplification of an RNA detector **probe** in the presence of the enzyme Q **beta replicase** . In a clinical matrix, the lower limit of detection of both the PCR and Q **beta replicase** assays was five elementary bodies. The Q **beta replicase** and PCR assays were quantitative over 10,000- and 1,000-fold ranges of organisms, respectively. Analysis of the effects of endocervical matrix on amplification was accomplished by examining 94 endocervical specimens by each technique. Both assays detected five of six culture-confirmed specimens as well as three culture-negative specimens. PCR inhibitors were detected in 13 specimens. The Q **beta replicase** assay, in contrast, showed no evidence of sample inhibition. The Q **beta replicase** and PCR assays should allow quantitative investigation of infections due to C. trachomatis. In addition, because it targets highly labile RNA, the Q **beta replicase** assay may facilitate investigations into the role of active persisting infection in culture-negative inflammatory conditions.

8/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08385575 95155589 PMID: 7531719
Novel, ultrasensitive, Q-**beta replicase** -amplified hybridization assay for detection of Chlamydia trachomatis.
Shah JS; Liu J; Smith J; Popoff S; Radcliffe G; O'Brien WJ; Serpe G; Olive DM; King W
GENE-TRAK Inc., Framingham, Massachusetts 01701.
Journal of clinical microbiology (UNITED STATES) Nov 1994, 32 (11) p2718-24, ISSN 0095-1137 Journal Code: HSH
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
A sensitive, nonisotopic hybridization assay termed "dual capture" is described. The assay rapidly and specifically detects very low levels of target nucleic acids and organisms. The assay is based on the principles of

sandwich hybridization, reversible target capture and Q-**Beta replicase** amplification. The assay can be completed in less than 4 h, and in the described model format, it detects Chlamydia trachomatis rRNA or rDNA. Up to 96 samples can be analyzed simultaneously. The assay employs two types of probes: a test-specific capture **probe**, which mediates the cycling of the target **probe** complex on and off derivatized magnetic beads, and a replicatable RNA detector molecule containing a sequence complementary to and adjacent to the capture **probe** site on the target. Following reversible target capture, detection of the signal is accomplished by replication of the detector molecule by Q-**Beta replicase** in the presence of propidium iodide. A specific assay signal can be detected from as few as 1,000 molecules above the background. In a limited study of 94 urogenital samples the assay detected five of the six culture-positive samples and did not detect the C. trachomatis target in 85 of the 88 culture-negative samples.

8/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08382027 95034822 PMID: 7524666
Evolution of host cell RNA into efficient template RNA by Q **beta replicase**: the origin of RNA in untemplated reactions.
Moody MD; Burg JL; DiFrancesco R; Lovern D; Stanick W; Lin-Goerke J; Mahdavi K; Wu Y; Farrell MP
Gene-Trak, Incorporated, Framingham, Massachusetts 01701.
Biochemistry (UNITED STATES) Nov 22 1994, 33 (46) p13836-47, ISSN 0006-2960 Journal Code: A0G
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Q **beta replicase** can replicate a single molecule of certain species of RNA to 10(14) copies in minutes. This replication ability has been used for in vitro studies of molecular evolution and is currently being utilized as a method of amplifying RNAs that contain **probe** sequences. It has been observed that Q **beta replicase** can produce replicatable RNA even in the absence of exogenously added template RNA. The origin of this RNA has been ascribed either to contamination with replicatable RNA or to an ability of Q **beta replicase** to synthesize RNA de novo from the nucleotides present in the reaction. Technologies that employ Q **beta replicase** require a thorough understanding of the conditions that lead to this so-called spontaneous RNA production. We have created an expression system and purification method with which we produce gram quantities of highly purified Q **beta replicase** , and we have identified reaction conditions that prevent the amplification of RNA in assays that do not contain added RNA. However, when these reaction conditions are relaxed, spontaneous RNA replication is seen in up to 100% of the assays. To understand the origin of this RNA, we have cloned several spontaneously produced RNAs. Sequence analysis of one of these RNAs shows that it arose by the evolution of Escherichia coli tRNA into a replicatable template and not by de novo synthesis from nucleoside triphosphates in the reaction.

8/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08282748 95055746 PMID: 7966321
Structure-mapping of the **hairpin ribozyme** . Magnesium-dependent folding and evidence for tertiary interactions within the **ribozyme**-substrate complex.
Butcher SE; Burke JM
Department of Microbiology and Molecular Genetics, Markey Center for Molecular Genetics, University of Vermont, Burlington 05405.
Journal of molecular biology (ENGLAND) Nov 18 1994, 244 (1) p52-63,

ISSN 0022-2836 Journal Code: J6V
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

We have used chemical modification analysis to **probe** the solution structure of the **hairpin ribozyme** . The modifying reagents dimethylsulfate, 1-cyclohexyl-N'-[2-(N-methylmorpholino) ethyl-carbodiimide -p-toluenesulfonate, kethoxal, diethylpyrocarbonate and (2,12-dimethyl-3,7, 11,17- tetraazabicyclo [11.3.1]heptadeca-1(17),2,11,13,15-pentaenato) nickel(II) perchlorate were used to **probe** functional groups that participate in Watson-Crick and non-canonical base-pairs. Our results confirm the existence of four short helices (3 to 6 bp) within the **ribozyme** -substrate complex, and demonstrate that one intramolecular helix (helix 4) is comprised of three base-pairs rather than the previously suggested five. In the absence of magnesium, the **ribozyme** is observed to fold into its secondary structure. Upon addition of magnesium, a striking difference in chemical modification is observed, particularly at sites within the **ribozyme** 's large internal loop (loop B) that are essential for catalytic function (bases 21 to 26). Moreover, magnesium-dependent folding clearly destabilizes an A-U base-pair in a region where a proposed bend is required to juxtapose the catalytically essential loops A and B. Upon addition of substrate, no changes are observed in the structure of helix 3, loop B or helix 4. However, strong protection of bases in the substrate-binding domain is observed, including those located across internal loop A. The modification data are consistent with the formation of a previously proposed tertiary structure motif within loop B that includes non-canonical G-A, A-U and A-A base-pairs, and that is identical with those identified by NMR analysis of loop E of 5 S rRNA and the sarcin/ricin loop of 28 S rRNA. Our results indicate that the **hairpin ribozyme** adopts a stable magnesium-dependent tertiary structure to which the substrate binds without inducing major conformational changes, and that substrate recognition is likely to involve non-canonical base-pairs between the **ribozyme** and substrate sequences adjacent to the cleavage site.

8/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08168162 94261427 PMID: 8202361
Probing the hammerhead **ribozyme** structure with ribonucleases.
Hodgson RA; Shirley NJ; Symons RH
Department of Plant Science, Waite Institute, University of Adelaide, Australia.
Nucleic acids research (ENGLAND) May 11 1994, 22 (9) p1620-5, ISSN 0305-1048 Journal Code: O8L
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

Susceptibility to RNase digestion has been used to **probe** the conformation of the hammerhead **ribozyme** structure prepared from chemically synthesised RNAs. Less than about 1.5% of the total sample was digested to obtain a profile of RNase digestion sites. The observed digestion profiles confirmed the predicted base-paired secondary structure for the hammerhead. Digestion profiles of both cis and trans hammerhead structures were nearly identical which indicated that the structural interactions leading to self-cleavage were similar for both systems. Furthermore, the presence or absence of Mg2+ did not affect the RNase digestion profiles, thus indicating that Mg2+ did not modify the hammerhead structure significantly to induce self-cleavage. The base-paired stems I and II in the hammerhead structure were stable whereas stem III, which was susceptible to digestion, appeared to be an unstable region. The single strand domains separating the stems were susceptible to digestion with the exception of sites adjacent to guanosines; GL2.1 in the stem II loop and G12 in the conserved GAAAC sequence, which separates stems II and III. The

absence of digesti[redacted] at GL2.1 in the stem II hai[redacted]n loop of the hammerhead complex [redacted]as maintained in uncomplexed **ribozyme** and in short oligonucleotides containing only the stem II **hairpin** region. In contrast, the G12 site became susceptible when the **ribozyme** was not complexed with its substrate. Overall the results are consistent with the role of Mg2+ in the hammerhead self-cleavage reaction being catalytic and not structural.

8/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07852679 93181192 PMID: 8441628
Folding of DNA substrate-**hairpin ribozyme** domains: use of deoxy 4-thiouridine as an intrinsic photolabel.
Dos Santos DV; Vianna AL; Fourrey JL; Favre A
Groupe de Photobiologie Moleculaire, Institut Jacques Monod, CNRS Universite Paris VII, France.
Nucleic acids research (ENGLAND) Jan 25 1993, 21 (2) p201-7, ISSN 0305-1048 Journal Code: O8L
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Hairpin ribozymes derived from (-)sTRSV RNA exhibit substantial cleavage activity when wobble GU base pairs are introduced in place of the AU pairs normally involved in helices I and II between substrate and **ribozyme** . This finding prompted us to synthesize by in vitro transcription a new **hairpin ribozyme**, active against a 14-mer substrate derived from a conserved HIV sequence. Interactions of the canonical and anti-HIV **hairpin** ribozymes with non cleavable DNA substrate analogues containing the photoaffinity **probe** deoxy-4-thiouridine (ds4U) at a single site were investigated. Upon near-UV light irradiation (365 nm), all these substrate analogues were covalently attached to **ribozyme** via single or multiple crosslinks. In contrast, no crosslinks were detected using either a DNA substrate analogue lacking ds4U or a ds4U containing oligomer unrelated to the substrate sequence. As expected, if the dissociation constant is in the range of 5-15 microM, the yield of crosslinked **ribozyme** increased markedly with increasing the substrate analogue concentration. The **ribozyme** residues involved in the crosslinks were determined by RNA sequencing. The pattern of crosslinks obtained with the two **ribozyme** systems provides additional evidence in support of the consensus secondary structure proposed for the **hairpin** domain. Minor alternative conformations were detected in the case of the (-)sTRSV system.

8/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07780227 91371617 PMID: 1893572
Polymerase chain reaction and Q **beta replicase** amplification.
Cahill P; Foster K; Mahan DE
Gene-Trak Systems, Framingham, MA 01701.
Clinical chemistry (UNITED STATES) Sep 1991, 37 (9) p1482-5, ISSN 0009-9147 Journal Code: DBZ
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The polymerase chain reaction (PCR) and Q **beta replicase** are two methods in which nucleic acid polymerases are used for amplification. Although these approaches share many similar problems concerning target contamination and **probe** specificity, they differ dramatically in their mechanisms of action and modes of application. The PCR method amplifies target sequences between two priming oligonucleotides and in essence amplifies a portion of the analyte. Q **beta replicase**,

on the other hand, amplifies a specific template molecule hybridized to target sequences and therefore amplifies a signal component of the system. For this reason, Q **beta replicase** amplification has applications in areas other than for the detection of nucleic acid sequences. The requirements for application and the advantages of both PCR and Q **beta replicase** amplification are reviewed.

8/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07510616 92276418 PMID: 1816250
DNA **probe** amplification methods.
Birkenmeyer LG; Mushahwar IK
Experimental Biology Research, Abbott Laboratories, North Chicago, Illinois 60064.
Journal of virological methods (NETHERLANDS) Nov-Dec 1991, 35 (2) p117-26, ISSN 0166-0934 Journal Code: HQR
Languages: ENGLISH
Document type: Journal Article; Review; Review, Tutorial
Record type: Completed

8/3,AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06976932 93173662 PMID: 1283911
Cross-ligation and exchange reaction of RNA catalyzed by **hairpin** ribozymes.
Komatsu Y; Koizumi M; Sekiguchi A; Ohtsuka E
Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan.
Nucleic acids symposium series (ENGLAND) 1992, (27) p43-4, ISSN 0261-3166 Journal Code: O8N
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The catalytic domain in the minus strand of the satellite RNA of tobacco ringspot virus (sTobRV(-)) assumes a **hairpin** -like secondary structure. This **ribozyme** catalyzes a cross-ligation reaction between substrate RNAs of different lengths. We constructed ribozymes to **probe** the activities of ligation and RNA fragment exchange.

8/3,AB/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06682334 91119253 PMID: 2278413
Amplified detection of viral nucleic acid at subattomole levels using Q **beta replicase**.
Pritchard CG; Stefano JE
Gene-Trak Systems, Framingham, Massachusetts 01701.
Annales de biologie clinique (FRANCE) 1990, 48 (7) p492-7, ISSN 0003-3898 Journal Code: 4ZS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
A sensitive, amplified assay for HIV-1 pol region RNA was developed using RNA probes which are replicated by the RNA-dependent RNA polymerase, Q **beta replicase** . A synthetic target RNA was hybridized in cell lysates prepared with guanidine thiocyanate with an RNA reporter **probe** and four deoxyoligonucleotide "capture" probes. The RNA reporter **probe** was a recombinant MDV RNA molecule generated by transcription from a cloned cDNA template. Capture probes are synthetic oligonucleotides that are complementary to the target nucleic acid and that bear 3' poly d(A) tails. The ternary hybrids (of target RNA with capture

probe and report[redacted] **probe**) were captured [redacted] oligo d(T)-derivatized p[redacted]magnetic particles by hybridization with the d(A) tails of the capture probes. Non-hybridized reporter probes were removed by washing and successively eluting and recapturing the ternary hybrids on fresh particles. After three cycles of elution and capture, the hybrids were eluted in a low ionic strength buffer and the MDV RNA reporter probes were amplified directly by Q **beta replicase**. Amplified product RNA was detected by fluorescence using propidium iodide. The assay detects one femtogram (600 molecules) of a synthetic target RNA containing the pol region of HIV-1. The complete assay takes about 2.5 hours.

8/3,AB/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06664136 91023629 PMID: 2221501
Amplifiable hybridization probes.
Kramer FR; Lizardi PM
Department of Molecular Genetics, Public Health Research Institute, New York 10016.
Annales de biologie clinique (FRANCE) 1990, 48 (6) p409-11, ISSN 0003-3898 Journal Code: 4ZS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Amplifiable hybridization probes enable the development of extremely sensitive clinical assays. These novel molecules consist of a **probe** sequence embedded within the sequences of a replicatable RNA. The molecules are first hybridized to target sequences in a conventional manner. The **probe** -target complexes are then isolated and the probes are released from their targets. The released probes are then amplified by incubation with the RNA-directed RNA polymerase, Q-**beta replicase**. The replicase copies the probes in a geometrically increasing manner: after each round of copying, the number of RNA molecules is twice the previous number. The doubling process is very rapid, resulting in as many as one billion copies of each molecule in 30 minutes. The amount of RNA that is made is large enough to be measured without using radioisotopes. Theoretically, these assays should be extraordinarily sensitive, since only one **probe** molecule is required to start the amplification process. In practice the sensitivity of the assays is limited by the presence of non hybridized probes that persist, despite extensive washing of the **probe** -target hybrids. Currently, the limit of detection is about 10,000 molecules of target. However, replicatable probes are now being prepared that include a "molecular switch", which is a region of the RNA that undergoes a conformational change when the **probe** sequence hybridizes to its target. Protocols are being developed that link signal generation to the state of this switch. The simplicity and speed of the enzymatic steps that are required facilitate automation of the assays.

8/3,AB/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05417319 89376806 PMID: 2673578
Quantitative assays based on the use of replicatable hybridization probes.
Lomeli H; Tyagi S; Pritchard CG; Lizardi PM; Kramer FR
Centro de Investigacion sobre Ingenieria Genetica y Biotecnologia, Universidad Nacional Autonoma de Mexico, Morelos.
Clinical chemistry (UNITED STATES) Sep 1989, 35 (9) p1826-31, ISSN 0009-9147 Journal Code: DBZ
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Amplifiable hybridization probes--molecules with a **probe** sequence

embedded within the sequence of a replicatable RNA--will promote the development of sensitive clinical assays. To demonstrate their utility, we prepared a recombinant RNA that contained a 30-nucleotide-long **probe** complementary to a conserved region of the pol gene in human immunodeficiency virus type 1 (HIV-1) mRNA. Test samples were prepared, each containing a different number of HIV-1 transcripts that served as simulated HIV-1 mRNA targets. Hybridizations were carried out in a solution containing the chaotropic salt, guanidine thiocyanate. **Probe**-target hybrids were isolated by reversible target capture on paramagnetic particles. The probes were then released from their targets and amplified by incubation with the RNA-directed RNA polymerase, Q **beta replicase** (EC 2.7.7.48). The replicase copied the probes in an exponential manner: after each round of copying, the number of RNA molecules doubled. The amount of RNA synthesized in each reaction (approximately 50 ng) was sufficient to measure without using radioisotopes. Kinetic analysis of the reactions demonstrated that the number of HIV-1 targets originally present in each sample could be determined by measuring the time it took to synthesize a particular amount of RNA (the longer the synthesis took, the fewer the number of targets originally present). The results suggest that clinical assays involving replicatable hybridization probes will be simple, accurate, sensitive, and automatable.

8/3,AB/26 (Item 26 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05416617 89370644 PMID: 2672619
In vitro amplification techniques for the detection of nucleic acids: new tools for the diagnostic laboratory.
Persing DH; Landry ML
Department of Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut 06510.
Yale journal of biology and medicine (UNITED STATES) Mar-Apr 1989, 62 (2) p159-71, ISSN 0044-0086 Journal Code: XR7
Languages: ENGLISH
Document type: Journal Article; Review; Review, Tutorial
Record type: Completed
The acceptance of nucleic acid probes as diagnostic tools for the clinical laboratory has been hampered by a number of factors, including laborious techniques and limited sensitivity. The focus of this review is on the recent development of amplification techniques to enhance the signal generated by nucleic acid-based detection systems. Three general areas are discussed: (1) amplification of target sequences using the polymerase chain reaction or the transcript amplification system, (2) amplification of the **probe** sequences using Q **beta replicase** , and (3) amplification of **probe**-generated signals with compound or "Christmas tree" probes. The hope of these new technologies is to simplify yet improve on the sensitivity of nucleic acid-based tests to enable them to attain a more prominent place in the diagnostic repertoire of the clinical laboratory.

8/3,AB/27 (Item 27 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

03253100 78012199 PMID: 269389
Conformational alteration of protein synthesis elongation factor EF-Tu by EF-Ts and by kirromycin.
Blumenthal T; Douglass J; Smith D
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Aug 1977, 74 (8) p3264-7, ISSN 0027-8424 Journal Code: PV3
Languages: ENGLISH
Document type: Journal Article

Record type: Completed
Alterations of the structure of EF-Tu have been investigated by using the rate of EF-Tu cleavage by trypsin as a conformational **probe**. The presence of EF-Ts bound to EF-Tu results in a 10-fold increase in the cleavage rate. The antibiotic kirromycin, which inhibits protein synthesis by virtue of its interaction with EF-Tu, mimics this effect of EF-Ts. Both kirromycin and EF-Ts also facilitate the exchange of free GDP with GDP bound to EF-Tu. The results suggest that EF-Ts and kirromycin induce a similar conformational change in EF-Tu, thereby "opening" the guanine nucleotide binding site. The trypsin-cleaved EF-Tu still can bind GDP and EF-Ts and can function in Qbeta replicase, but it no longer spontaneously renatures following denaturation in urea.

8/3,AB/28 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13420281 BIOSIS NO.: 200200049102
Selective amplification system using Q-**beta replicase**
AUTHOR: Stefano J E
AUTHOR ADDRESS: Hopkinton, Mass.**USA
JOURNAL: Official Gazette of the United States Patent and Trademark Office Patents 1190 (3):p2039-2040 Sept. 17, 1996
ISSN: 0098-1133
DOCUMENT TYPE: Patent
RECORD TYPE: Citation
LANGUAGE: English
1996

8/3,AB/29 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12985687 BIOSIS NO.: 200100192836
Time-resolved hydroxyl-radical footprinting of RNA using Fe(II)-EDTA.
AUTHOR: Hampel Ken J; Burke John M(a)
AUTHOR ADDRESS: (a)Markey Center for Molecular Genetics, Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT, 05405: John.Burke@uvm.edu**USA
JOURNAL: Methods (Orlando) 23 (3):p233-239 March, 2001
MEDIUM: print
ISSN: 1046-2023
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Chemical footprinting methods have been used extensively to **probe** the structures of biologically important RNAs at nucleotide resolution. One of these methods, hydroxyl-radical footprinting, has recently been employed to study the kinetics of RNA folding. Hydroxyl radicals can be generated by a number of different methods, including Fe(II)-EDTA complexes, synchrotron radiation, and peroxynitrous acid disproportionation. The latter two methods have been used for kinetic studies of RNA folding. We have taken advantage of rapid hydroxyl-radical generation by Fe(II)-EDTA-hydrogen peroxide solutions to develop a benchtop method to study folding kinetics of RNA complexes. This technique can be performed using commercially available chemicals, and can be used to accurately define RNA folding rate constants slower than 6 min-1. Here we report the method and an example of time-resolved footprinting on the **hairpin ribozyme**, a small endoribonuclease and RNA ligase.

0006-2960 Journal [redacted]e: A0G
Contract/Grant No.: AI29892, AI, NIAID; AI30534, AI, NIAID
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

Most RNA molecules that are endowed with catalytic activity function in the form of ribonucleoproteins within cells. These complexes are frequently large, poorly defined, and difficult to study. As a model system to study biological catalysis by ribonucleoproteins, we have modified the **hairpin ribozyme** by inserting an RNA structure that serves as a binding site for bacteriophage R17 coat protein in the form of an extension to **ribozyme** helix 4, which lies at the periphery of the catalytic domain. In the absence of protein, we find that incorporation of the protein-binding domain increases the catalytic efficiency of the **hairpin ribozyme** by 2-fold for the cleavage reaction and 16-fold for the **ligation** reaction. This increase in activity correlates with an increase in the proportion of molecules which fold into the active tertiary structure, as measured by a UV cross-linking assay. Mobility-shift and filter-binding assays of complex formation show that R17 coat protein binds to the chimeric **ribozyme** with a dissociation constant essentially identical to that of the isolated protein-binding domain; no binding of the protein to the unmodified **ribozyme** could be detected. The kinetics of cleavage and **ligation** reactions are not altered by the presence of saturating concentrations of coat protein, and competition studies demonstrate that the protein remains bound to the **ribozyme** throughout the catalytic cycle. These studies establish that the **hairpin ribozyme** can be engineered to function efficiently in the form of a ribonucleoprotein in vitro and will serve as the basis for future experimentation to understand mechanisms of protein modulation of catalytic RNA activity, and to introduce other protein-binding domains, for example, HIV-1 rev-binding and tar elements, which may be useful for influencing subcellular localization, regulating intracellular activity, or generating ribozymes that also function as "decoys" in antiviral applications.

2/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09338737 97298064 PMID: 9153212
Kinetic mechanism of the **hairpin ribozyme**. Identification and characterization of two nonexchangeable conformations.
Esteban JA; Banerjee AR; Burke JM
Markey Center for Molecular Genetics, Department of Microbiology and Molecular Genetics, The University of Vermont, Burlington, Vermont 05405, USA.
Journal of biological chemistry (UNITED STATES) May 23 1997, 272 (21) p13629-39, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

To investigate the relationship between RNA folding and **ribozyme** catalysis, we have carried out a detailed kinetic analysis of four structural derivatives of the **hairpin ribozyme**. Optimal and suboptimal (wild-type) substrate sequences were studied in conjunction with stabilization of helix 4, which supports formation of the catalytic core. Pre-steady-state and steady-state kinetic studies strongly support a model in which each of the **ribozyme** variants partitions between two major conformations leading to active and inactive **ribozyme**. substrate complexes. Reaction rates for cleavage, **ligation** , and substrate binding to both **ribozyme** conformations were determined. **Ligation** rates (3 min-1) were typically 15-fold greater than cleavage rates (0.2 min-1), demonstrating that the **hairpin ribozyme** is an efficient RNA ligase. On the other hand, substrate binding is very rapid (kon = 4 x 10(8) M-1 min-1), and the **ribozyme**. substrate complex is

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File 5:Biosis Previews(R) 1969-2002/Feb W2
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7843 HAIRPIN
4617 RIBOZYME
70202 LIGAT?
S1 53 HAIRPIN AND RIBOZYME AND LIGAT?
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...examined 50 records (50)
...completed examining records
S2 28 RD (unique items)
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2/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12748982 21564075 PMID: 11707414
Functional involvement of G8 in the **hairpin ribozyme** cleavage mechanism.
Pinard R; Hampel K J; Heckman J E; Lambert D; Chan P A; Major F; Burke J M
Department of Microbiology and Molecular Genetics, The University of Vermont, 306 Stafford Hall, Burlington, VT 05405, USA.
EMBO journal (England) Nov 15 2001, 20 (22) p6434-42, ISSN 0261-4189 Journal Code: 8208664
Contract/Grant No.: AI 44186, AI, NIAID
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The catalytic determinants for the cleavage and **ligation** reactions mediated by the **hairpin ribozyme** are integral to the polyribonucleotide chain. We describe experiments that place G8, a critical guanosine, at the active site, and point to an essential role in catalysis. Cross-linking and modeling show that formation of a catalytic complex is accompanied by a conformational change in which N1 and O6 of G8 become closely apposed to the scissile phosphodiester. UV cross-linking, hydroxyl-radical footprinting and native gel electrophoresis indicate that G8 variants inhibit the reaction at a step following domain association, and that the tertiary structure of the inactive complex is not measurably altered. Rate-pH profiles and fluorescence spectroscopy show that protonation at the N1 position of G8 is required for catalysis, and that modification of O6 can inhibit the reaction. Kinetic solvent isotope analysis suggests that two protons are transferred during the rate-limiting

step, consistent with rate-limiting cleavage chemistry involving concerted deprotonation of the attacking 2'-OH and protonation of the 5'-O leaving group. We propose mechanistic models that are consistent with these data, including some that invoke a novel keto-enol tautomerization.

2/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11780876 21535549 PMID: 11680850
Investigation of adenosine base ionization in the **hairpin ribozyme** by nucleotide analog interference mapping.
Ryder S P; Oyelere A K; Padilla J L; Klostermeier D; Millar D P; Strobel S A
Yale University, Department of Molecular Biophysics and Biochemistry, New Haven, Connecticut 06520-8114, USA.
RNA (United States) Oct 2001, 7 (10) p1454-63, ISSN 1355-8382 Journal Code: CHB
Contract/Grant No.: GM 58873, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: In Process
Tertiary structure in globular RNA folds can create local environments that lead to pKa perturbation of specific nucleotide functional groups. To assess the prevalence of functionally relevant adenosine-specific pKa perturbation in RNA structure, we have altered the nucleotide analog interference mapping (NAIM) approach to include a series of a phosphorothioate-tagged adenosine analogs with shifted N1 pKa values. We have used these analogs to analyze the **hairpin ribozyme**, a small self-cleaving/**ligating** RNA catalyst that is proposed to employ a general acid-base reaction mechanism. A single adenosine (A10) within the **ribozyme** active site displayed an interference pattern consistent with a functionally significant base ionization. The exocyclic amino group of a second adenosine (A38) contributes substantially to **hairpin** catalysis, but ionization of the nucleotide does not appear to be important for activity. Within the **hairpin ribozyme** crystal structure, A10 and A38 line opposite edges of a solvent-excluded cavity adjacent to the 5'-OH nucleophile. The results are inconsistent with the model of **ribozyme** chemistry in which A38 acts as a general acid-base catalyst, and suggest that the **hairpin ribozyme** uses an alternative mechanism to achieve catalytic rate enhancement that utilizes functional groups within a solvent-excluded cleft in the **ribozyme** active site.

2/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11446644 21314164 PMID: 11421363
Imaging of single **hairpin** ribozymes in solution by atomic force microscopy.
Fay MJ; Walter NG; Burke JM
Markey Center for Molecular Genetics, Department of Microbiology and Molecular Genetics, The University of Vermont, Burlington 05405, USA.
RNA (United States) Jun 2001, 7 (6) p887-95, ISSN 1355-8382 Journal Code: CHB
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The **hairpin ribozyme** is a short endonucleolytic RNA motif isolated from a family of related plant virus satellite RNAs. It consists of two independently folding domains, each comprising two Watson-Crick helices flanking a conserved internal loop. The domains need to physically interact (dock) for catalysis of site-specific cleavage and **ligation** reactions. Using tapping-mode atomic force microscopy in aqueous buffer solution, we were able to produce high quality images of individual

hairpin ribozyme molecules with extended terminal helices. Three RNA constructs with either the essential cleavage site guanosine or a detrimental adenosine substitution and with or without a 6-nt insertion to confer flexibility to the interdomain hinge show structural differences that correlate with their ability to form the active docked conformation. The observed contour lengths and shapes are consistent with previous bulk-solution measurements of the transient electric dichroism decays for the same RNA constructs. The active docked construct appears as an asymmetrically docked conformation that might be an indication of a more complicated docking event than a simple collapse around the interdomain hinge.

2/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11424411 21293193 PMID: 11399066
Hairpin ribozymes with four-way helical junctions mediate intracellular RNA **ligation**.
Yadava RS; Choi AJ; Lebruska LL; Fedor MJ
Department of Molecular Biology and The Skaggs Institute for Chemical Biology, The Scripps Research Institute MB35, 10550 N. Torrey Pines Road, La Jolla, CA 92037, USA.
Journal of molecular biology (England) Jun 15 2001, 309 (4) p893-902, ISSN 0022-2836 Journal Code: J6V
Contract/Grant No.: GM46422, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Virtually all RNA-mediated reactions require transitions among alternative RNA conformations. The complexity of biological reactions can obscure specific conformational changes in vivo and important features of the intracellular environment are difficult to reproduce in vitro. However, simple RNA self-cleavage and **ligation** reactions offer a unique opportunity to measure the kinetics and equilibria of specific RNA conformational transitions directly in living cells. **Hairpin** ribozymes that incorporate the natural four-way helical junction self-cleave rapidly in vivo, but only when cleavage products dissociate rapidly. Cleavage rates fall when cleavage products remain bound in stable base-paired helices, providing evidence that bound products undergo re-**ligation** . These results provide the first detailed kinetic description of an intracellular **ribozyme** reaction that includes cleavage, **ligation** and product dissociation rates. Kinetic and equilibrium parameters measured in vivo correspond well, but not perfectly, with values measured for the same reactions in vitro under conditions that approximate an intracellular ionic environment. Copyright 2001 Academic Press.

2/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11355771 21194705 PMID: 11297441
A conformational change in the "loop E-like" motif of the **hairpin ribozyme** is coincidental with domain docking and is essential for catalysis.
Hampel KJ; Burke JM
Department of Microbiology and Molecular Genetics, University of Vermont, Burlington 05405, USA.
Biochemistry (United States) Mar 27 2001, 40 (12) p3723-9, ISSN 0006-2960 Journal Code: A0G
Contract/Grant No.: AI 44186, AI, NIAID
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

The catalysis of [redacted]te-specific RNA cleavage and **ation** by the **hairpin ribozyme** [redacted]quires the formation of a rtiary interaction between two independently folded internal loop domains, A and B. Within the B domain, a tertiary structure has been identified, known as the loop E motif, that has been observed in many naturally occurring RNAs. One characteristic of this motif is a partial cross-strand stack of a G residue on a U residue. In a few cases, including loop B of the **hairpin ribozyme** , this unusual arrangement gives rise to photoreactivity. In the **hairpin**, G21 and U42 can be UV cross-linked. Here we show that docking of the two domains correlates very strongly with a loss of UV reactivity of these bases. The rate of the loss of photoreactivity during folding is in close agreement with the kinetics of interdomain docking as determined by hydroxyl-radical footprinting and fluorescence resonance energy transfer (FRET). Fixing the structure of the complex in the cross-linked form results in an inability of the two domains to dock and catalyze the cleavage reaction, suggesting that the conformational change is essential for catalysis.

2/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10543797 20181858 PMID: 10715200
Structure and function of the **hairpin ribozyme**.
Fedor MJ
Department of Molecular Biology and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, MB35, 10550 N. Torrey Pines Road, La Jolla, CA 92037, USA. mfedor@scripps.edu
Journal of molecular biology (ENGLAND) Mar 24 2000, 297 (2) p269-91, ISSN 0022-2836 Journal Code: J6V
Contract/Grant No.: RO1GM46422, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article; Review; Review, Academic
Record type: Completed
The **hairpin ribozyme** belongs to the family of small catalytic RNAs that cleave RNA substrates in a reversible reaction that generates 2',3'-cyclic phosphate and 5'-hydroxyl termini. The **hairpin** catalytic motif was discovered in the negative strand of the tobacco ringspot virus satellite RNA, where **hairpin ribozyme**-mediated self-cleavage and **ligation** reactions participate in processing RNA replication intermediates. The self-cleaving **hairpin**, hammerhead, hepatitis delta and Neurospora VS RNAs each adopt unique structures and exploit distinct kinetic and catalytic mechanisms despite catalyzing the same chemical reactions. Mechanistic studies of **hairpin ribozyme** reactions provided early evidence that, like protein enzymes, RNA enzymes are able to exploit a variety of catalytic strategies. In contrast to the hammerhead and Tetrahymena **ribozyme** reactions, **hairpin**-mediated cleavage and **ligation** proceed through a catalytic mechanism that does not require direct coordination of metal cations to phosphate or water oxygens. The **hairpin ribozyme** is a better ligase than it is a nuclease while the hammerhead reaction favors cleavage over **ligation** of bound products by nearly 200-fold. Recent structure-function studies have begun to yield insights into the molecular bases of these unique features of the **hairpin ribozyme**. Copyright 2000 Academic Press.

2/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10485153 20090964 PMID: 10623557
The kinetic mechanism of the **hairpin ribozyme** in vivo: influence of RNA helix stability on intracellular cleavage kinetics.
Donahue CP; Yadava RS; Nesbitt SM; Fedor MJ
Department of Molecular Biology, Skaggs Institute for Chemical Biology, The Scripps Research Institute, MB35, 10550 N. Torrey Pines Rd, La Jolla,

CA, 92037, USA.
Journal of molecular biology (ENGLAND) Jan 21 2000 295 (3) p693-707
, ISSN 0022-2836 Journal Code: J6V
Contract/Grant No.: RO1 GM46422, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The relationship between **hairpin ribozyme** structure, and cleavage and **ligation** kinetics, and equilibria has been characterized extensively under a variety of reaction conditions in vitro. We developed a quantitative assay of **hairpin ribozyme** cleavage activity in yeast to learn how structure-function relationships defined for RNA enzymes in vitro relate to RNA-mediated reactions in cells. Here, we report the effects of variation in the stability of an essential secondary structure element, H1, on intracellular cleavage kinetics. H1 is the base-paired helix formed between **ribozyme** and 3' cleavage product RNAs. H1 sequences with fewer than three base-pairs fail to support full activity in vitro or in vivo, arguing against any significant difference in the stability of short RNA helices under in vitro and intracellular conditions. Under standard conditions in vitro that include 10 mM MgCl(2), the internal equilibrium between cleavage and **ligation** of **ribozyme**-bound products favors **ligation** . Consequently, ribozymes with stable H1 sequences display sharply reduced self-cleavage rates, because cleavage is reversed by rapid re-**ligation** of bound products. In contrast, ribozymes with as many as 26 base-pairs in H1 continue to self-cleave at maximum rates in vivo. The failure of large products to inhibit cleavage could be explained if intracellular conditions promote rapid product dissociation or shift the internal equilibrium to favor cleavage. Model experiments in vitro suggest that the internal equilibrium between cleavage and **ligation** of bound products is likely to favor cleavage under intracellular ionic conditions. Copyright 2000 Academic Press.

2/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10418783 20022426 PMID: 10554775
The **hairpin ribozyme**. Discovery, mechanism, and development for gene therapy.
Shippy R; Lockner R; Farnsworth M; Hampel A
Department of Biological Sciences, Northern Illinois University, DeKalb 60115, USA.
Molecular biotechnology (UNITED STATES) Aug 1999, 12 (1) p117-29, ISSN 1073-6085 Journal Code: B97
Languages: ENGLISH
Document type: Journal Article; Review; Review, Tutorial
Record type: Completed
The **hairpin ribozyme** is a member of a family of small RNA endonucleases, which includes hammer-head, human hepatitis delta virus, Neurospora VS, and the lead-dependent catalytic RNAs. All these catalytic RNAs reversibly cleave the phosphodiester bond of substrate RNA to generate 5'-hydroxyl and 2',3'-cyclic phosphate termini. Whereas the reaction products from family members are similar, large structural and mechanistic differences exist. Structurally the **hairpin ribozyme** has two principal domains that interact to facilitate catalysis. The **hairpin ribozyme** uses a catalytic mechanism that does not require metals for cleavage or **ligation** of substrate RNA. In this regard it is presently unique among RNA catalysts. Targeting rules for cleavage of substrate have been determined and required bases for catalysis have been identified. The **hairpin ribozyme** has been developed and used for gene therapy and was the first **ribozyme** to be approved for human clinical trials.

2/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10264536 99370079 PMID: 10438622
Nucleotide analog interference mapping of the **hairpin ribozyme** : implications for secondary and tertiary structure formation.
Ryder SP; Strobel SA
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520-8114, USA.
Journal of molecular biology (ENGLAND) Aug 13 1999, 291 (2) p295-311 , ISSN 0022-2836 Journal Code: J6V
Contract/Grant No.: GM07223, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The **hairpin ribozyme** is a small, naturally occurring RNA capable of folding into a distinct three-dimensional structure and catalyzing a specific phosphodiester transfer reaction. We have adapted a high throughput screening procedure entitled nucleotide analog interference mapping (NAIM) to identify functional groups important for proper folding and catalysis of this **ribozyme**. A total of 18 phosphorothioate-tagged nucleotide analogs were used to determine the contribution made by individual ribose 2'-OH and purine functional groups to the **hairpin ribozyme ligation** reaction. Substitution with 2'-deoxy-nucleotide analogs disrupted activity at six sites within the **ribozyme** , and a unique interference pattern was observed at each of the 11 conserved purine nucleotides. In most cases where such information is available, the NAIM data agree with the previously reported single-site substitution results. The interference patterns are interpreted in comparison to the isolated loop A and loop B NMR structures and a model of the intact **ribozyme** . These data provide biochemical evidence in support of many, but not all, of the non-canonical base-pairs observed by NMR in each loop, and identify the functional groups most likely to participate in the tertiary interface between loop A and loop B. These groups include the 2'-OH groups of A10, G11, U12, C25, and A38, the exocyclic amine of G11, and the minor groove edge of A9 and A24. The data also predict non-A form sugar pucker geometry at U39 and U41. Based upon these results, a revised model for the loop A tertiary interaction with loop B is proposed. This work defines the chemical basis of purine nucleotide conservation in the **hairpin ribozyme**, and provides a basis for the design and interpretation of interference suppression experiments. Copyright 1999 Academic Press.

2/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10250913 99389566 PMID: 10460159
Tertiary structure stabilization promotes **hairpin ribozyme ligation**.
Fedor MJ
Department of Molecular Biology, The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, California 92037, USA. mfedor@scripps.edu
Biochemistry (UNITED STATES) Aug 24 1999, 38 (34) p11040-50, ISSN 0006-2960 Journal Code: A0G
Contract/Grant No.: RO1 GM46422, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The **hairpin ribozyme** catalyzes a reversible RNA cleavage reaction that participates in processing intermediates of viral satellite RNA replication in plants. A minimal **hairpin ribozyme** consists of two helix-loop-helix segments. These segments associate noncoaxially in the active folded structure in a way that brings catalytically important loop nucleotides into close proximity. The **hairpin ribozyme** in

the satellite RNA of Tobacco Ringspot Virus assembles in the context of a four-way helical junction. Recent physical characterization of **hairpin ribozyme** structures using fluorescence resonance energy transfer demonstrated enhanced stability of the folded structure in the context of a four-way helical junction compared to minimal **hairpin ribozyme** variants. Analysis of the functional consequences of this modification of the helical junction has revealed two changes in the **hairpin ribozyme** kinetic mechanism. First, ribozymes with a four-way helical junction bind 3' cleavage products with much higher affinity than minimal **hairpin** ribozymes, evidence that tertiary interactions within the folded structure contribute to product binding energy. Second, the balance between **ligation** and cleavage shifts in favor of **ligation**. The enhanced **ligation** activity of **hairpin** ribozymes that contain a four-way helical junction supports the notion that tertiary structure stability is a major determinant of the **hairpin ribozyme** proficiency as a ligase and illustrates the link between RNA structure and biological function.

2/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10079684 99158807 PMID: 10047478

The internal equilibrium of the **hairpin ribozyme** : temperature, ion and pH effects.

Nesbitt SM; Erlacher HA; Fedor MJ

Department of Molecular Biology and The Skaggs Institute for Chemical Biology, The Scripps Research Institute MB35, 10550 North Torrey Pines, La Jolla, CA, 92037, USA.

Journal of molecular biology (ENGLAND) Mar 5 1999, 286 (4) p1009-24, ISSN 0022-2836 Journal Code: J6V

Contract/Grant No.: RO1 GM46422, GM, NIGMS

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The **hairpin ribozyme** reversibly cleaves phosphodiesters of RNA substrates to generate products with 5' hydroxyl and 2',3'-cyclic phosphate termini. We previously found that the rate constant for **ligation** is tenfold faster than the rate constant for cleavage under standard conditions. The hammerhead **ribozyme** catalyzes the same reactions but is reported to favor cleavage relative to **ligation** by more than 100-fold under the same conditions. To explore the basis for this difference, we examined the influence of temperature, ions and pH on the **hairpin ribozyme** internal equilibrium. Under the same conditions, the loss of entropy associated with **ligation** is less for the **hairpin** than for the hammerhead **ribozyme**, consistent with the notion that a more rigid **hairpin** structure undergoes a smaller decrease in dynamics upon **ligation** than the more flexible hammerhead structure. Increased salt and reduced temperature shift the equilibrium toward **ligation** while pH has little effect, suggesting that conditions that stabilize RNA structure tend to promote **ligation**. The **hairpin ribozyme** appears to take up at least one tri- or divalent cation or two monovalent cations upon **ligation** . The efficiency with which different cations promote **ligation** depends strongly on valence and, less strongly, on ionic radius or electronegativity. This pattern of cation selectivity suggests that cations promote **ligation** through delocalized electrostatic shielding, perhaps interacting with a region of especially high charge density in the **ligated ribozyme**. Changes in ionic conditions produce large but compensating changes in enthalpy and entropy for cleavage and **ligation**. Thus, in addition to any increase in **ribozyme** dynamics associated with cleavage, re-organization of associated cations contributes significantly to **hairpin ribozyme** thermodynamics.

2/3,AB/12 (Item from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09768999 98211946 PMID: 9545249
Tertiary structure formation in the **hairpin ribozyme** monitored by fluorescence resonance energy transfer.
Walter NG; Hampel KJ; Brown KM; Burke JM
Markey Center for Molecular Genetics, Department of Microbiology and Molecular Genetics, The University of Vermont, Burlington, VT 05405, USA.
EMBO journal (ENGLAND) Apr 15 1998, 17 (8) p2378-91, ISSN 0261-4189 Journal Code: EMB
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The complex formed by the **hairpin ribozyme** and its substrate consists of two independently folding domains which interact to form a catalytic structure. Fluorescence resonance energy transfer methods permit us to study reversible transitions of the complex between open and closed forms. Results indicate that docking of the domains is required for both the cleavage and **ligation** reactions. Docking is rate-limiting for **ligation** (2 min-1) but not for cleavage, where docking (0.5 min-1) precedes a rate-limiting conformational transition or slow-reaction chemistry. Strikingly, most modifications to the RNA (such as a G+1A mutation in the substrate) or reaction conditions (such as omission of divalent metal ion cofactors) which inhibit catalysis do so by preventing docking. This demonstrates directly that mutations and modifications which inhibit a step following substrate binding are not necessarily involved in catalysis. An improved kinetic description of the catalytic cycle is derived, including specific structural transitions.

2/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09697079 98155834 PMID: 9494730
Screening for important base identities in the **hairpin ribozyme** by in vitro selection for cleavage.
Siwkowski A; Humphrey M; De-Young MB; Hampel A
Northern Illinois University, DeKalb, USA.
BioTechniques (UNITED STATES) Feb 1998, 24 (2) p278-84, ISSN 0736-6205 Journal Code: AN3
Contract/Grant No.: R01AI29870, AI, NIAID
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Random mutagenesis followed by an in vitro selection procedure was shown to be capable of identifying important bases of the **hairpin ribozyme** for cleavage of an RNA target sequence. The selection scheme enriched the RNA population for those molecules capable of efficient site-specific self-cleavage in the absence of **ligation**. Cleavable mutants were selected for all positions in loop 4 except for position A38, supporting the notion that A38 is an important base in the **hairpin ribozyme** . This has been confirmed by direct mutagenesis, validating the utility of this procedure. Thus, the method developed and reported here has utility for the selection of efficient **hairpin** ribozymes capable of highly efficient cleavage of a substrate RNA without a requirement for **ribozyme**-catalyzed **ligation** , conditions desired for many applications of catalytic RNA such as gene therapy.

2/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09588539 97428598 PMID: 9281529

An unusual pH-independent and metal-ion-independent mechanism for **hairpin ribozyme** catalysis.
Nesbitt S; Hegg LA; Fedor MJ
Department of Biochemistry and Molecular Biology, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, MA 01655, USA.
Chemistry & biology (ENGLAND) Aug 1997, 4 (8) p619-30, ISSN 1074-5521 Journal Code: CNA
Contract/Grant No.: GM 46422, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
BACKGROUND: **Hairpin** ribozymes (RNA enzymes) catalyze the same chemical reaction as ribonuclease A and yet RNAs do not usually have functional groups analogous to the catalytically essential histidine and lysine sidechains of protein ribonucleases. Some RNA enzymes appear to recruit metal ions to act as Lewis acids in charge stabilization and metal-bound hydroxide for general base catalysis, but it has been reported that the **hairpin ribozyme** functions in the presence of metal ion chelators. This led us to investigate whether the **hairpin ribozyme** exploits a metal-ion-independent catalytic strategy. RESULTS: Substitution of sulfur for nonbridging oxygens of the reactive phosphate of the **hairpin ribozyme** has small, stereospecific and metal-ion-independent effects on cleavage and **ligation** mediated by this **ribozyme** . Cobalt hexammine, an exchange-inert metal complex, supports full **hairpin ribozyme** activity, and the **ribozyme** 's catalytic rate constants display only a shallow dependence on pH. CONCLUSIONS: Direct metal ion coordination to phosphate oxygens is not essential for **hairpin ribozyme** catalysis and metal-bound hydroxide does not serve as the general base in this catalysis. Several models might account for the unusual pH and metal ion independence: **hairpin** cleavage and **ligation** might be limited by a slow conformational change; a pH-independent or metal-cation-independent chemical step, such as breaking the 5' oxygen-phosphorus bond, might be rate determining; or finally, functional groups within the **ribozyme** might participate directly in catalytic chemistry. Whichever the case, the **hairpin ribozyme** appears to employ a unique strategy for RNA catalysis.

2/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09544929 97348439 PMID: 9204449
Ligation of RNA molecules by the **hairpin ribozyme**.
Berzal-Herranz A; Burke JM
Instituto de Parasitologia y Biomedicina (CSIC), Granada, Spain.
Methods in molecular biology (UNITED STATES) 1997, 74 p349-55, ISSN 1064-3745 Journal Code: BU3
Contract/Grant No.: AI29892, AI, NIAID; AI30534, AI, NIAID
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

2/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09497528 95298784 PMID: 7779820
An improved version of the **hairpin ribozyme** functions as a ribonucleoprotein complex.
Sargueil B; Pecchia DB; Burke JM
Department of Microbiology and Molecular Genetics, University of Vermont, Burlington 05405, USA.
Biochemistry (UNITED STATES) Jun 13 1995, 34 (23) p7739-48, ISSN

very stable (KD < 25 pM; koff < 0.01 min-1). Stabilization of helix 4 increases the proportion of RNA molecules folded into the active conformation, and enhances substrate association and **ligation** rates. These effects can be explained by stabilization of the catalytic core of the **ribozyme** . Rigorous consideration of conformational isomers and their intrinsic kinetic properties was necessary for development of a kinetic scheme for the **ribozyme**-catalyzed reaction.

2/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09310135 97280788 PMID: 9135115
Identification of functional domains in the self-cleaving Neurospora VS **ribozyme** using damage selection.
Beattie TL; Collins RA
Department of Molecular and Medical Genetics, University of Toronto, Ontario, Canada.
Journal of molecular biology (ENGLAND) Apr 11 1997, 267 (4) p830-40, ISSN 0022-2836 Journal Code: J6V
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Varkud Satellite (VS) RNA contains a small self-cleaving RNA motif that is distinct in its sequence and secondary structure from the hammerhead, **hairpin** , and hepatitis delta virus ribozymes, which are found in other natural RNAs. We have used a base specific chemical damage selection (modification interference) assay to identify functionally important nucleotides and structural elements in VS RNA. Many modified bases interfered with self-cleavage and most of these clustered at helix junctions, certain internal loops, and in a long-range pseudoknot; these correspond to previously determined sites of magnesium-dependent protection from chemical modification. The clustering suggests that these bases are important not only for a large number of individual interactions, but because they form a smaller number of structural elements that are important for activity. Modification of bases in other single-stranded regions, which did not exhibit magnesium-dependent protection, generally did not interfere with activity, suggesting that some of these regions might be dispensable for function. Surprisingly, we found a separate cluster of bases whose modification significantly enhanced cleavage. These bases appear to form a structural element that naturally attenuates the self-cleavage reaction. In natural VS RNA this attenuator structure may affect the cleavage/**ligation** equilibrium by inhibiting circle re-opening, thereby helping to maintain the RNA in a circular form, which is the predominant form of VS RNA in vivo. Taken together, the results of the damage selection experiments localize the catalytic core of VS RNA to a small subset of the previously determined minimal contiguous sequence.

2/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08875019 96183887 PMID: 8642668
A pseudoknot **ribozyme** structure is active in vivo and required for hepatitis delta virus RNA replication.
Jeng KS; Daniel A; Lai MM
Howard Hughes Medical Institute, University of Southern California School of Medicine, Los Angeles 90033-1054, USA.
Journal of virology (UNITED STATES) Apr 1996, 70 (4) p2403-10, ISSN 0022-538X Journal Code: KCV
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The ribozymes of hepatitis delta virus (HDV) have so far been studied primarily in vitro. Several structural models for HDV ribozymes based on